[0016] Typically, purified peptides are only marginally stable in an aqueous state and undergo chemical and physical degradation resulting in a loss of biological activity during processing and storage. Additionally, peptide compositions in aqueous solution undergo hydrolysis, such as deamidation and peptide bond cleavage. These effects represent a serious problem for therapeutically active peptides which are intended to be administered to humans within a defined dosage range based on biological activity.

[0017] Administration of purified peptides remains a promising treatment strategy for many diseases that affect the human population. However, the ability of the therapeutic peptibody to remain a stable pharmaceutical composition over time in a variety of storage conditions and then be effective for patients *in vivo* has not been addressed. Thus, there remains a need in the art to provide therapeutic peptibodies in stable formulations that are useful as therapeutic agents for the treatment of diseases and disorders.

SUMMARY

[0018] The present disclosure provides formulations useful for lyophilization of therapeutic peptibodies, resulting in a highly stable therapeutic peptibody product. The stable therapeutic peptibody product is useful as a therapeutic agent in the treatment of individuals suffering from disorders or conditions that can benefit from the administration of the therapeutic peptibody.

[0018a] In one aspect, the disclosure provides a stable lyophilized therapeutic peptibody composition comprising a buffer, a bulking agent, a stabilizing agent, and a surfactant, wherein: the buffer is 10 mM histidine; the pH is 5.0; the bulking agent is 4% w/v mannitol; the stabilizing agent is 2% w/v sucrose; the surfactant is 0.004% w/v polysorbate-20; and the therapeutic peptibody is a dimer including a structure of the following formula: Fc-GGGGG-IEGPTLRQWLAARA-GGGGGGGG-IEGPTLRQWLAARA (SEQ ID NO: 1017).**[0018b]** In another aspect, the disclosure provides a kit for preparing an aqueous pharmaceutical composition, comprising a first container having the stable lyophilized therapeutic peptibody composition as described herein, and a second container having a physiologically acceptable solvent for the lyophilized composition.

[0019] In one example, the disclosure provides a lyophilized therapeutic peptibody composition comprising a buffer, a bulking agent, a stabilizing agent, and optionally a surfactant; wherein the buffer is comprised of a pH buffering agent in a range of about 5 mM to about 20 mM and wherein the pH is in a range of about 3.0 to about 8.0;

wherein the bulking agent is at a concentration of about 0% to about 4.5% w/v; wherein the stabilizing agent is at a concentration of about 0.1% to about 20% w/v; wherein the surfactant is at a concentration of about 0.004% to about 0.4% w/v; and wherein the therapeutic peptibody comprises a structure set out in Formula I,

Formula I: $[(X^1)_a\text{-}F^1\text{-}(X^2)_b]\text{-}(L^1)_c\text{-}WSP_d$

wherein:

F^1 is an Fc domain;

X^1 is selected from

$P^1\text{-}(L^2)_e\text{-}$

P^2-$(L^3)_f$ -P^1-$(L^2)_e$-

P^3-$(L^4)_g$-P^2 -$(L^3)_f$ -P^1-$(L^2)_e$- and

P^4-$(L^5)_h$-P^3-$(L^4)_g$-P^2-$(L^3)_f$-P^1-$(L^2)_e$ -

X^2 is selected from:

-$(L^2)_e$-P^1,

-$(L^2)_e$-P^1-$(L^3)_f$ -P^2,

-$(L^2)_e$-P^1-$(L^3)_f$-P^2-$(L^4)_g$-P^3, and

-$(L^2)_e$-P^1-$(L^3)_f$-P^2-$(L^4)_g$ -P^3-$(L^5)_h$-P^4

wherein P^1, P^2, P^3, and P^4 are each independently sequences of pharmacologically active peptides;

L^1, L^2, L^3, L^4, and L^5 are each independently linkers;

a, b, c, e, f, g, and h are each independently 0 or 1,

provided that at least one of a and b is 1;

d is 0, 1, or greater than 1; and

WSP is a water soluble polymer, the attachment of which is effected at any reactive moiety in F^1.

[0020] In another example, the therapeutic peptibody comprises a structure set out in Formula II

Formula II: $[X^1\text{-}F^1]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the C-terminus of X^1, and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0021] In still another example, the therapeutic peptibody comprises a structure set out in Formula III

Formula III: $[F^1\text{-}X^2]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the N-terminus of X^2, and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0022] In yet another example, the therapeutic peptibody comprises a structure set out in Formula IV

Formula IV: $[F^1\text{-}(L^1)_e\text{-}P^1]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the N-terminus of $-(L^1)_c\text{-}P^1$ and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0023] In another example, the the therapeutic peptibody comprises a structure set out in Formula V

Formula V: $[F^1\text{-}(L^1)_e\text{-}P^1\text{-}(L^2)_f\text{-}P^2]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the N-terminus of $-L^1\text{-}P^1\text{-}L^2\text{-}P^2$ and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0024] In another example, the therapeutic peptibody is a multimer or dimer. In another example, an aforementioned composition is provided wherein P^1, P^2, P^3 and/or P^4 are independently selected from a peptide set out in any one of Tables 4 through 38. In a related example, P^1, P^2, P^3 and/or P^4 have the same amino acid sequence. In another example, the Fc domain is set out in SEQ ID NO:1. In another example, WSP is PEG. In still another example, the Fc domain is et out in SEQ ID NO:1 and WSP is PEG. In another example, the PEG has a molecular weight of between about 2 kDa and 100 kDa, or between 6 kDa and 25 kDa. In another example, the composition comprises at least 50%, 75%, 85%, 90%, or 95% PEGylated therapeutic peptibody.

[0025] In yet another example of the disclosure, an aforementioned composition is provided wherein the pH buffering agent is selected from the group consisting of glycine, histidine, glutamate, succinate, phosphate, acetate, and aspartate. In yet another example of the disclosure, an aforementioned composition is provided wherein the bulking agent selected from the group consisting of mannitol, glycine, sucrose, dextran, polyvinylpyrolidone, carboxymethylcellulose, lactose, sorbitol, trehalose, or xylitol.

[0026] In yet another example of the disclosure, an aforementioned composition is provided wherein the stabilizing agent selected from the group consisting of sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, mannitol, sorbitol, glycine, arginine HCL, poly-hydroxy compounds, including polysaccharides such as dextran, starch, hydroxyethyl starch, cyclodextrins, N-methyl pyrollidene, cellulose and hyaluronic acid, sodium chloride.

[0027] In yet another example of the disclosure, an aforementioned composition is provided wherein the surfactant selected from the group consisting of sodium lauryl sulfate, dioctyl sodium sulfosuccinate, dioctyl sodium sulfonate, chenodeoxycholic acid, N-lauroylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate, sodium deoxycholate, glycodeoxycholic acid sodium salt, benzalkonium chloride or benzethonium chloride, cetylpyridinium chloride monohydrate, hexadecyltrimethylammonium bromide, CHAPS, CHAPSO, SB3-10, SB3-12, digitonin,

Triton X-100, Triton X-114, lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 40, 50 and 60, glycerol monostearate, polysorbate 20, 40, 60, 65 and 80, soy lecithin, DOPC, DMPG, DMPC, and DOPG; sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. In yet another example of the disclosure, an aforementioned composition is provided wherein the therapeutic peptibody concentration is between about 0.25 mg/mL and 250 mg/mL.

[0028] In another example of the disclosure, an aforementioned composition is provided wherein the pH buffering agent is 10 mM histidine and wherein the pH is 5.0; wherein the bulking agent is 4% w/v mannitol; wherein the stabilizing agent is 2% w/v sucrose; and wherein the surfactant is 0.004% w/v polysorbate-20. In another example, the aforementioned composition is provided wherein P^1 comprises a sequence set forth in Table 6. In yet another example of the disclosure, an aforementioned composition is provided wherein the therapeutic peptibody concentration is 0.5 mg/mL. In another example, the therapeutic peptibody is any one of SEQ ID NO:993, SEQ ID NO:994, SEQ ID NO:995, SEQ ID NO:996, SEQ ID NO:997, SEQ ID NO:998, SEQ ID NO:999, SEQ ID NO:1000, SEQ ID NO:1001, SEQ ID NO:1002, SEQ ID NO:1003, SEQ ID NO:1004, SEQ ID NO:1005, SEQ ID NO:1006, SEQ ID NO:1007, SEQ ID NO:1008, SEQ ID NO:1009, SEQ ID NO:1010, SEQ ID NO:1011, SEQ ID NO:1012, SEQ ID NO:1013, SEQ ID NO:1014, SEQ ID NO:1015, SEQ ID NO:1016, or SEQ ID NO:1017.

[0029] In yet another example of the disclosure, an aforementioned composition is provided wherein the pH buffering agent is 10 mM histidine and wherein the pH is 7.0; wherein the bulking agent is 4% w/v mannitol; wherein the stabilizing agent is 2% w/v sucrose; and wherein the surfactant is 0.01% w/v polysorbate-20. In another example, the aforementioned composition is provided wherein P^1 comprises a sequence set forth in Table 32. In yet another example of the disclosure, an aforementioned composition is provided wherein the therapeutic peptibody concentration is 30 mg/mL.

[0030] In still another example of the disclosure, an aforementioned composition is provided wherein the pH buffering agent is 20 mM histidine and wherein the pH is 5.0; wherein the bulking agent is 3.3% w/v mannitol; wherein the stabilizing agent is 2% w/v sucrose; and wherein the surfactant is 0.01% w/v polysorbate-20. In another example, the aforementioned composition is provided wherein P^1 comprises a sequence set forth in Table 4. In yet another example of the disclosure, an aforementioned composition is provided wherein the therapeutic peptibody concentration is 100 mg/mL.

[0031] In still another example of the disclosure, an aforementioned composition is provided wherein the pH buffering agent is 10 mM histidine and wherein the pH is 5.0; wherein the bulking agent is 2.5% w/v mannitol; and wherein the stabilizing agent is 3.5% w/v sucrose. In another example, the aforementioned composition is provided wherein P^1 comprises a sequence set forth in Table 31. In yet another example of the disclosure, an aforementioned composition is provided wherein the therapeutic peptibody concentration is 30 mg/mL.

[0032] In another example of the disclosure, an aforementioned composition is provided wherein the composition is selected from the group consisting of: a) 10 mM histidine, pH 4.7, 4% mannitol and 2% sucrose, with and without 0.004% polysorbate-20; b) 10 mM histidine, pH 5**,** 4% mannitol and 2% sucrose, with and without 0.004% polysorbate-20; c) 10 mM glutamate, pH 4.5, 4% mannitol and 2% sucrose with and without 0.004% polysorbate-20; d) 10 mM succinate, pH 4.5, 4% mannitol and 2% sucrose, 0.004% polysorbate-20; e) 10 mM glutamate, pH 4.5, 9% sucrose, 0.004% polysorbate-20; f) 10 mM glutamate, pH 4.5, 4% mannitol, 2% sucrose, 1% hydroxyethyl starch, 0.004% polysorbate-20; g) 5 mM glutamate, pH 4.5, 2% mannitol, 1% sucrose, 0.004% polysorbate-20; and h) 10 mM glutamate, pH 4.5, 4% mannitol, 2% trehalose, 0.004% polysorbate-20. In another example, the aforementioned composition is provided wherein P^1 comprises a sequence set forth in Tables 21-24. In still another example, the aforementioned composition is provided wherein the therapeutic peptibody concentration is selected from the group consisting of 1, 30, 85, and 100 mg/mL.

[0033] The present disclosure also contemplates methods of making lyophilized therapeutic peptibodies of the present disclosure. In one example, the disclosure provides a method for making a lyophilized therapeutic peptibody comprising the steps of: a) preparing a solution of a buffer, a bulking agent, a stabilizing agent, and a optionally surfactant; wherein the buffer is comprised of a pH buffering agent in a range of about 5 mM to about 20 mM and wherein the pH is in a range of about 3.0 to about 8.0; wherein the bulking agent is at a concentration of about 2.5% to about 4% w/v; wherein the stabilizing agent is at a concentration of about 0.1% to about 5% w/v; wherein the surfactant is at a concentration of about 0.004% to about 0.04% w/v; and b) lyophilizing the therapeutic peptibody; wherein the therapeutic peptibody comprises a structure set out in Formula I,

Formula I: $[(X^1)_a\text{-}F^1\text{-}(X^2)_b]\text{-}(L^1)_c\text{-}WSP_d$

wherein:

F^1 is an Fc domain;
X^1 is selected from

P^1-$(L^2)_e$-

P^2-$(L^3)_f$ -P^1-$(L^2)_e$-

P^3-$(L^4)_g$-P^2 -$(L^3)_f$ -P^1-$(L^2)_e$- and

P^4-$(L^5)_h$-P^3-$(L^4)_g$-P^2-$(L^3)_f$-P^1-$(L^2)_e$ -

X^2 is selected from:

-$(L^2)_e$-P^1,

-$(L^2)_e$-P^1-$(L^3)_f$ -P^2,

-$(L^2)_e$-P^1-$(L^3)_f$-P^2-$(L^4)_g$-P^3, and

-$(L^2)_e$-P^1-$(L^3)_f$-P^2-$(L^4)_g$ -P^3-$(L^5)_h$-P^4

wherein P^1, P^2, P^3, and P^4 are each independently sequences of pharmacologically active peptides;

L^1, L^2, L^3, L^4, and L^5 are each independently linkers;

a, b, c, e, f, g, and h are each independently 0 or 1,

provided that at least one of a and b is 1;

d is 0, 1, or greater than 1; and

WSP is a water soluble polymer, the attachment of which is effected at any reactive moiety in F^1.

[0034] In another example, the aforementioned method is provided wherein thee therapeutic peptibody comprises a structure set out in Formula II

Formula II: $[X^1\text{-}F^1]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the C-terminus of X^1, and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0035] In another example, the aforementioned method is provided wherein the therapeutic peptibody comprises a structure set out in Formula III

Formula III: $[F^1\text{-}X^2]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the N-terminus of X^2, and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0036] In another example, the aforementioned method is provided wherein the therapeutic peptibody comprises a structure set out in Formula IV

Formula IV: $[F^1\text{-}(L^1)_e\text{-}P^1]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the N-terminus of $-(L^1)_c\text{-}P^1$ and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0037] In another example, the aforementioned method is provided wherein the therapeutic peptibody comprises a structure set out in Formula V

Formula V: $[F^1\text{-}(L^1)_e\text{-}P^1\text{-}(L^2)_f\text{-}P^2]\text{-}(L^1)_c\text{-}WSP_d$

wherein the Fc domain is attached at the N-terminus of $-L^1\text{-}P^1\text{-}L^2\text{-}P^2$ and zero, one or more WSP is attached to the Fc domain, optionally through linker L^1.

[0038] In another example, the aforementioned method is provided wherein the therapeutic peptibody is a multimer or dimer. In another example, the P^1, P^2, P^3 and/or P^4 are independently selected from a peptide set out in any one of Tables 4 through 38. In another example, the P^1, P^2, P^3 and/or P^4 have the same amino acid sequence. In another example, the Fc domain is set out in SEQ ID NO:1. In another example, WSP is PEG. In another example, the Fc domain is set out in SEQ ID NO:1 and WSP is PEG. In another example, PEG has a molecular weight of between about 2 kDa and 100 kDa or between about 6 kDa and 25 kDa. In another example, the aforementioned method is provided wherein the composition comprises at least 50%, 75%, 85%, 90%, or 95% PEGylated therapeutic peptibody.

[0039] In another example, the aforementioned method is provided wherein the pH buffering agent is selected from the group consisting of glycine, histidine, glutamate, succinate, phosphate, acetate, and aspartate. In another example, the aforementioned method is provided wherein the bulking agent selected from the group consisting of mannitol, glycine, sucrose, dextran, polyvinylpyrolidone, carboxymethylcellulose, lactose, sorbitol, trehalose, or xylitol. In another example, the aforementioned method is provided wherein the stabilizing agent selected from the group consisting of sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, mannitol, sorbitol, glycine, arginine HCL, poly-hydroxy compounds, including polysaccharides such as dextran, starch, hydroxyethyl starch, cyclodextrins, N-methyl pyrollidene, cellulose and hyaluronic acid, sodium chloride.

[0040] In another example, the aforementioned method is provided wherein the surfactant selected from the group consisting of sodium lauryl sulfate, dioctyl sodium sulfosuccinate, dioctyl sodium sulfonate, chenodeoxycholic acid, N-lauroylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate,

sodium deoxycholate, glycodeoxycholic acid sodium salt, benzalkonium chloride or benzethonium chloride, cetylpyridinium chloride monohydrate, hexadecyltrimethylammonium bromide, CHAPS, CHAPSO, SB3-10, SB3-12, digitonin, Triton X-100, Triton X-114, lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 40, 50 and 60, glycerol monostearate, polysorbate 20, 40, 60, 65 and 80, soy lecithin, DOPC, DMPG, DMPC, and DOPG; sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. In another example, the aforementioned method is provided wherein the therapeutic peptibody concentration is between about 0.25 mg/mL and 250 mg/mL.

[0041] In another example, an aforementioned method is provided wherein the pH buffering agent is 10 mM histidine and wherein the pH is 5.0; wherein the bulking agent is 4% w/v mannitol; wherein the stabilizing agent is 2% w/v sucrose; and wherein the surfactant is 0.004% w/v polysorbate-20. In another example, the aforementioned method is provided wherein P^1 comprises a sequence set forth in Table 6. In another example, the aforementioned method is provided wherein the therapeutic peptibody concentration is 0.5 mg/mL.

[0042] In another example, an aforementioned method is provided wherein the pH buffering agent is 10 mM histidine and wherein the pH is 7.0; wherein the bulking agent is 4% w/v mannitol; wherein the stabilizing agent is 2% w/v sucrose; and wherein the surfactant is 0.01% w/v polysorbate-20. In another example, the aforementioned method is provided wherein P^1 comprises a sequence set forth in Table 32. In another example, the aforementioned method is provided wherein the therapeutic peptibody concentration is 30 mg/mL.

[0043] In another example, an aforementioned method is provided wherein the pH buffering agent is 20 mM histidine and wherein the pH is 5.0; wherein the bulking agent is 3.3% w/v mannitol; wherein the stabilizing agent is 2% w/v sucrose; and wherein the surfactant is 0.01% w/v polysorbate-20. In another example, the aforementioned method is provided wherein P^1 comprises a sequence set forth in Table 4. In another example, the aforementioned method is provided wherein the therapeutic peptibody concentration is 100 mg/mL.

[0044] In another example, an aforementioned method is provided wherein the pH buffering agent is 10 mM histidine and wherein the pH is 5.0; wherein the bulking agent is 2.5% w/v mannitol; and wherein the stabilizing agent is 3.5% w/v sucrose. In another example, the aforementioned method is provided wherein P^1 comprises a sequence set

forth in Table 31. In another example, the aforementioned method is provided wherein the therapeutic peptibody concentration is 30 mg/mL.

[0045] In another example of the disclosure, an aforementioned method is provided wherein the composition is selected from the group consisting of: a) 10 mM histidine, pH 4.7, 4% mannitol and 2% sucrose, with and without 0.004% polysorbate-20; b) 10 mM histidine, pH 5**,** 4% mannitol and 2% sucrose, with and without 0.004% polysorbate-20; c) 10 mM glutamate, pH 4.5, 4% mannitol and 2% sucrose with and without 0.004% polysorbate-20; d) 10 mM succinate, pH 4.5, 4% mannitol and 2% sucrose, 0.004% polysorbate-20; e) 10 mM glutamate, pH 4.5, 9% sucrose, 0.004% polysorbate-20; f) 10 mM glutamate, pH 4.5, 4% mannitol, 2% sucrose, 1% hydroxyethyl starch, 0.004% polysorbate-20; g) 5 mM glutamate, pH 4.5, 2% mannitol, 1% sucrose, 0.004% polysorbate-20; and h) 10 mM glutamate, pH 4.5, 4% mannitol, 2% trehalose, 0.004% polysorbate-20. In another example, the aforementioned method is provided wherein P^1 comprises a sequence set forth in Tables 21-24. In still another example, the aforementioned method is provided wherein the therapeutic peptibody concentration is selected from the group consisting of 1, 30, 85, and 100 mg/mL.

[0046] In another example, an aforementioned method is provided further comprising, prior to lyophilization, the steps of: b) adjusting the pH of the solution to a pH between about 4.0 and about 8.0; c) preparing a solution containing the therapeutic peptibody; d) buffer exchanging the solution of step (c) into the solution of step (b); e) adding an appropriate amount of a surfactant; and f) lyophilizing the mixture from step (e).

[0047] In another example, the aforementioned method is provided wherein a method for preparing a reconstituted therapeutic peptibody composition is provided comprising the steps of: a) lyophilizing an aforementioned therapeutic peptibody composition; and b) reconstituting the lyophilized therapeutic peptibody composition.

[0048] In another example, a kit for preparing an aqueous pharmaceutical composition is provided comprising a first container having an aforementioned lyophilized therapeutic peptibody composition, and a second container having a physiologically acceptable solvent for the lyophilized composition.

DETAILED DESCRIPTION OF THE INVENTION

Definition of terms

[0049] The term "comprising," with respect to a peptide compound, means that a compound may include additional amino acids on either or both of the amino or carboxy termini of the

What is claimed is:

1. A stable lyophilized therapeutic peptibody composition comprising a buffer, a bulking agent, a stabilizing agent, and a surfactant, wherein:

the buffer is 10 mM histidine;

the pH is 5.0;

the bulking agent is 4% w/v mannitol;

the stabilizing agent is 2% w/v sucrose;

the surfactant is 0.004% w/v polysorbate-20; and

the therapeutic peptibody is a dimer including a structure of the following formula:

Fc-GGGGG-IEGPTLRQWLAARA-GGGGGGGG-IEGPTLRQWLAARA (SEQ ID NO: 1017).

2. The composition as claimed in claim 1, wherein the Fc domain is set out in SEQ ID NO:1.

3. The composition as claimed in claim 1, wherein the concentration of therapeutic peptibody is between 0.25 mg/mL and 250 mg/mL.

4. The composition as claimed in claim 1, wherein the concentration of therapeutic peptibody is 0.5 mg/mL.

5. A kit for preparing an aqueous pharmaceutical composition, comprising a first container having the stable lyophilized therapeutic peptibody composition as claimed in claim 1, and a second container having a physiologically acceptable solvent for the lyophilized composition.

6. The composition as claimed in claim 2, wherein the peptibody has methionine at the N-terminus.