formulations. Previous methods of preparing glucagon powder formulations suitable for nasal administration are disclosed in WO2016/133863.

There exists a need for alternative methods for preparing peptide powder formulations, in particular glucagon or glucagon analog powder formulations. In particular, there is a need for methods which reduce or eliminate aggregation of the peptide in aqueous solution. By reducing, or, preferably, eliminating aggregation, the final powder formulation will retain a very high percentage of active peptide, which is highly advantageous. Preferably, the method results in an aqueous solution prior to drying which is physically and chemically stable for an extended period of time, for example up to 24 hours. This extended stability makes the process much more amenable to large scale manufacture. There is furthermore a need for a method which results in a final powder formulation having a long shelf-life, preferably up to about 24 months.

Accordingly, the present invention provides an improved and cost effective method of reducing aggregation of a peptide during the manufacture of a powder formulation. This method incorporates a double filtration step. One such peptide used in the present invention is glucagon or a glucagon analog. The powder formulations prepared according to the present method are particularly suitable for nasal administration.

Summary

In an aspect of the present disclosure, there is provided a method for preparing a peptide powder formulation comprising the steps of:

a. forming a first mixture of an acid, a phospholipid surfactant, and a cyclodextrin in an aqueous carrier;
b. subjecting the first mixture to a first filtration step wherein the filter comprises a membrane with a pore size of ~~about~~ 0.4 µm to ~~about~~ 0.5 µm;
c. adding a peptide to the first filtration product to form a second mixture, and subjecting the second mixture to a second filtration step wherein the filter comprises a membrane with a pore size of ~~about~~ 0.4 µm to ~~about~~ 0.5 µm; and
d. drying the second filtration product to form a solid formulation and processing the solid formulation to produce a final powder formulation.

In another ~~aspect~~ example of the present disclosure, there is provided a powder formulation prepared by the method disclosed herein.

Description

In accordance with one aspect of the invention, a method for preparing a peptide powder formulation is provided. This method comprises the steps of:

a. forming a first mixture of an acid, a phospholipid surfactant, and a cyclodextrin in an aqueous carrier;
b. subjecting the first mixture to a first filtration step wherein the filter comprises a membrane with a pore size of about 0.4 µm to about 0.5 µm;
c. adding a peptide to the first filtration product to form a second mixture, and subjecting the second mixture to a second filtration step wherein the filter comprises a membrane with a pore size of about 0.4 µm to about 0.5 µm; and
d. drying the second filtration product to form a solid formulation and processing the solid formulation to produce a final powder formulation.

In an embodiment, the peptide is glucagon or a glucagon analog. In particular, it is glucagon.

WE CLAIM:

1. A method for preparing a peptide powder formulation comprising the steps of:
    a. forming a first mixture of an acid, a phospholipid surfactant, and a cyclodextrin in an aqueous carrier;
    b. subjecting the first mixture to a first filtration step wherein the filter comprises a membrane with a pore size of ~~about~~ 0.4 µm to ~~about~~ 0.5 µm;
    c. adding a peptide to the first filtration product to form a second mixture, and subjecting the second mixture to a second filtration step wherein the filter comprises a membrane with a pore size of ~~about~~ 0.4 µm to ~~about~~ 0.5 µm; and
    d. drying the second filtration product to form a solid formulation and processing the solid formulation to produce a final powder formulation.
2. The method of claim 1 wherein the peptide is [Lys^{17,18}, Glu^{21}] glucagon ~~or a glucagon analog~~.
3. The method of claim ~~2~~ 1 wherein the peptide is glucagon.
4. The method of any one of claim 1 to 3 wherein the surfactant, the cyclodextrin and the peptide together constitute between ~~about~~ 1.5% and ~~about~~ 3% by weight of the second mixture.
5. The method of claim 4 wherein the surfactant, the cyclodextrin and the peptide together constitute ~~about~~ 2% by weight of the second mixture.
6. The method of claim 4 wherein the surfactant, the cyclodextrin and the peptide together constitute ~~about~~ 2.5% by weight of the second mixture.
7. The method of any one of claims 1 to 6 wherein the membrane in both the first and the second filtration steps comprises a polyvinylidene difluoride (PVDF) membrane.
8. The method of any one of claims 1 to 7 wherein the membrane in both the first and the second filtration steps comprises a pore size of ~~about~~ 0.45 µm.
9. The method of any one of claims 1 to 8 wherein the acid is citric acid or acetic acid.
10. The method of claim 9 wherein the acid is acetic acid.
11. The method of claim 10 wherein the acetic acid is at a concentration of 1M.
12. The method of any one of claims 1 to 11 wherein the surfactant is dodecylphosphocholine, didecylphosphatidylcholine, lysolauroylphosphatidylcholine, dioctanoylphosphatidylcholine, or dilauroylphosphatidylglycerol.
13. The method of claim 12 wherein the surfactant is dodecylphosphocholine.

14. The method of any one of claims 1 to 13 wherein the cyclodextrin is α-cyclodextrin, β-cyclodextrin, hydroxypropyl β-cyclodextrin, or γ-cyclodextrin.

15. The method of claim 14 wherein the cyclodextrin is α-cyclodextrin.

~~15.~~16. The method of claim 14 wherein the cyclodextrin is β-cyclodextrin.

~~16.~~17. The method of claim 1, wherein the peptide is glucagon and the method comprises the steps of:

a. forming a first mixture of acetic acid, dodecylphosphocholine, and β-cyclodextrin in an aqueous carrier;

b. subjecting the first mixture to a first filtration step wherein the filter comprises a membrane with a pore size of ~~about~~ 0.4 µm to ~~about~~ 0.5 µm;

c. adding glucagon to the first filtration product to form a second mixture, and subjecting the second mixture to a second filtration step wherein the filter comprises a membrane with a pore size of ~~about~~ 0.4 µm to ~~about~~ 0.5 µm; and

d. drying the second filtration product to form a solid formulation and processing the solid formulation to produce a final powder formulation.

~~17.~~18. The method of claim ~~16~~ 17 wherein the dodecylphosphocholine, the β-cyclodextrin and the glucagon together constitute ~~about~~ 2.5% by weight of the second mixture.

~~18.~~19. The method of claim ~~16~~ 17 or claim ~~17~~ 18 wherein the membrane in both the first and the second filtration steps comprises a PVDF membrane.

~~19.~~20. The method of any one of claims ~~16~~ 17 to ~~18~~ 19 wherein the membrane in both the first and the second filtration steps comprises a pore size of ~~about~~ 0.45 µm.

~~20.~~21. The method of any one of claims ~~16~~ 17 to ~~19~~ 20 wherein the acetic acid is at a concentration of 1M.

~~21.~~22. The method of any one of claims ~~16~~ 17 to ~~20~~ 21 wherein greater than 98% of the glucagon in the final powder formulation is non-aggregated glucagon as measured by reversed phase-HPLC.

~~22. A powder formulation prepared by the method according to any one of claims 16 to 21.~~

~~23. A powder formulation according to claim 22 wherein greater than 98% of the glucagon in the final powder formulation is non-aggregated glucagon as measured by reversed phase-HPLC.~~

23. The method according to any one of claims 17 to 22, wherein the final powder formulation comprises glucagon, dodecylphosphocholine (DPC), and β-cyclodextrin in a weight ratio of 10:10:80 (glucagon: DPC: β-cyclodextrin).