aggregation over time, it is preferred to prepare the emulsion at the time of use. Thus, a method and an apparatus used for preparing such emulsion has been proposed (Patent Literature 2 and Patent Literature 3).

[Prior Art Literatures]

[Patent Literatures]

[0005]

[Patent Literature 1] WO 2015/060235

[Patent Literature 2] WO 2007/083763

[Patent Literature 3] JP Patent No. 5,629,882

[Summary]

[0005a] In one aspect, the present disclosure provides a water-in-oil (w/o) emulsion formulation containing 3 types of peptides having 4 linked CTL epitopes each comprising 4 CTL epitope peptides linked via linkers in an aqueous phase, wherein the 3 types of peptides having 4 linked CTL epitopes are a peptide represented by Formula (I); a peptide represented by Formula (II); and a peptide represented by Formula (III):

A-(L)-B-(L)-C-(L)-PEP2 (I);

D-(L)-E-(L)-F-(L)-PEP4 (II); and

G-(L)-H-(L)-I-(L)-PEP10 (III),

in Formulae (I), (II), and (III),

(L) represents an arginine dimer;

A, B, and C are each independently selected from the group consisting of PEP7 PEP8, and PEP13, except for a combination of A representing PEP7 and B representing PEP8; D, E, and F are each independently selected from the group consisting of PEP5, PEP6, and PEP9; and G, H, and I are each independently selected from the group consisting of PEP1, PEP15, and PEP18, and the peptide represented by Formula (I) additionally have an arginine tetramer;

PEP1 represents a CTL epitope peptide represented by SEQ ID NO: 1;

PEP2 represents a CTL epitope peptide represented by SEQ ID NO: 2;

PEP4 represents a CTL epitope peptide represented by SEQ ID NO: 4;

PEP5 represents a CTL epitope peptide represented by SEQ ID NO: 5;

PEP6 represents a CTL epitope peptide represented by SEQ ID NO: 6;

PEP7 represents a CTL epitope peptide represented by SEQ ID NO: 7;

PEP8 represents a CTL epitope peptide represented by SEQ ID NO: 8;

PEP9 represents a CTL epitope peptide represented by SEQ ID NO: 9;

PEP10 represents a CTL epitope peptide represented by SEQ ID NO: 10;

PEP13 represents a CTL epitope peptide represented by SEQ ID NO: 13;

PEP15 represents a CTL epitope peptide represented by SEQ ID NO: 15;

PEP18 represents a CTL epitope peptide represented by SEQ ID NO: 18,

wherein the concentration of 3 types of peptides having 4 linked CTL epitopes in the aqueous phase in total is 3 to 27 mg/ml.

[0005b] In another aspect, the present disclosure provides a method for preparing the emulsion formulation according to the disclosure comprising the steps (i) and (ii):

(i) a step of injecting a part of an aqueous phase comprising peptides having 4 linked CTL epitopes into an oil phase comprising an oil adjuvant to prepare a mixture; and

(ii) a step of allowing the mixture to reciprocate between syringes connected via a syringe connector,

wherein the step (i) and the step (ii) are repeated to obtain an emulsion formulation comprising the total amount of the oil phase and the aqueous phase, and wherein the amount of a part of the aqueous phase is 1/10 to 1/2 fold (v/v) relative to the amount of the oil phase comprising an oil adjuvant.

[0005c] In another aspect, the present disclosure provides a use of an apparatus for preparing a water-in-oil emulsion formulation according to the disclosure, wherein the apparatus is composed of:

a second syringe 2;

a syringe connector 5 connected to the second syringe 2;

a three-way stopcock 4 connected to the syringe connector 5; and

a first syringe 1 and a third syringe 3 further connected to the three-way stopcock 4,

wherein the apparatus enables production of a water-in-oil emulsion formulation by adding an aqueous

phase filled in the third syringe 3 to the oil phase filled in the first syringe 1 or the second syringe 2 and allowing the resulting mixture to reciprocate between the first syringe 1 and the second syringe 2 via the syringe connector 5.

[0005d] In another aspect, the present disclosure provides a use of a kit for preparing an w/o emulsion formulation according to the disclosure, wherein the kit comprises peptides having 4 linked CTL epitopes each comprising CTL epitope peptides linked via linkers, an oil adjuvant, and the apparatus according to the disclosure.

[0005e] In another aspect, the present disclosure provides a use of a solution of peptides having 4 linked CTL epitopes in the manufacture of the water-in-oil (w/o) emulsion formulation according to the disclosure.

[Objects to Be Attained by the Invention]

[0006]

A method for preparing an emulsion formulation of the peptide having 4 linked CTL epitopes as disclosed in WO 2015/060235 is disclosed in the publication thereof. However, the disclosure concerns preparation of an emulsion formulation of a single type of peptide having 4 linked CTL epitopes, and WO 2015/060235 does not disclose any emulsion formulation containing 3 types of peptides having 4 linked CTL epitopes according to the present invention. Therefore, the first object of the present invention is to provide a w/o emulsion formulation that stably contains 3 types of peptides having 4 linked CTL epitopes.

[0007]

When preparing the emulsion formulation containing 3 types of peptides having 4 linked CTL epitopes according to the present invention in accordance with a standard method of emulsion preparation, the procedure for preparation is time-consuming, preparation may end up in a failure, and workers are seriously burdened in clinical situations such as hospitals where emulsion formulations are to be prepared at the time of use, although such problems were not known. The present inventors prepared the emulsion formulation containing 3 types of peptides having 4 linked CTL epitopes according to the present invention in accordance with a standard method of emulsion preparation and found these problems. Accordingly, the second object of the present invention is to provide a method for efficiently preparing the w/o emulsion formulation described above.

a second syringe 2;

a syringe connector 5 connected to the second syringe 2;

a three-way stopcock 4 connected to the syringe connector 5; and

a first syringe 1 and a third syringe 3 further connected to the three-way stopcock 4,

wherein the apparatus enables production of a water-in-oil emulsion formulation by adding an aqueous phase filled in the third syringe 3 to the oil phase filled in the first syringe 1 or the second syringe 2 and allowing the resulting mixture to reciprocate between the first syringe 1 and the second syringe 2 via the syringe connector 5.

[11] The apparatus according to [10], wherein the volumes of the first, the second, and the third syringes are each 3 ml or less.

[12] The apparatus according to [10] or [11], wherein an inner diameter of the syringe connector 5 through which the mixture passes is within a range from 0.8 to 1.7 mm.

[13] A kit used for preparing an w/o emulsion containing peptides having 4 linked CTL epitopes, which comprises peptides having 4 linked CTL epitopes each comprising 4 CTL epitope peptides linked via linkers, an oil adjuvant, and the apparatus according to any of [10] to [12].

[0010]

The present invention also relates to the following aspects ~~under items [15] to [18]~~.

[14] A method for treatment of tumor of a patient comprising administering the water-in-oil (w/o) emulsion formulation according to any of [1] to [7] to the patient.

[15] The water-in-oil (w/o) emulsion formulation according to any of [1] to [7] for use in the treatment of tumor.

[16] Use of a solution of peptides having 4 linked CTL epitopes in the manufacture of the water-in-oil (w/o) emulsion formulation according to any of [1] to [7].

[17] The apparatus according to any of [10] to [12] or the kit according to [13], which is used for preparing the water-in-oil (w/o) emulsion formulation for the treatment of tumor at the time of use.

[18] The apparatus according to any of [10] to [12] or the kit according to [13], wherein the water-in-oil (w/o) emulsion formulation is the formulation according to any of [1] to [7].

[19] A method for treatment of tumor of a patient comprising:

Claims

[1] A water-in-oil (w/o) emulsion formulation containing 3 types of peptides having 4 linked CTL epitopes each comprising 4 CTL epitope peptides linked via linkers in an aqueous phase, wherein the 3 types of peptides having 4 linked CTL epitopes are a peptide represented by Formula (I); a peptide represented by Formula (II); and a peptide represented by Formula (III):

A-(L)-B-(L)-C-(L)-PEP2 (I);

D-(L)-E-(L)-F-(L)-PEP4 (II); and

G-(L)-H-(L)-I-(L)-PEP10 (III),

in Formulae (I), (II), and (III),

(L) represents an arginine dimer;

A, B, and C are each independently selected from the group consisting of PEP7, PEP8, and PEP13, except for a combination of A representing PEP7 and B representing PEP8; D, E, and F are each independently selected from the group consisting of PEP5, PEP6, and PEP9; and G, H, and I are each independently selected from the group consisting of PEP1, PEP15, and PEP18, and the peptide represented by Formula (I) additionally have an arginine tetramer;

PEP1 represents a CTL epitope peptide represented by SEQ ID NO: 1;

PEP2 represents a CTL epitope peptide represented by SEQ ID NO: 2;

PEP4 represents a CTL epitope peptide represented by SEQ ID NO: 4;

PEP5 represents a CTL epitope peptide represented by SEQ ID NO: 5;

PEP6 represents a CTL epitope peptide represented by SEQ ID NO: 6;

PEP7 represents a CTL epitope peptide represented by SEQ ID NO: 7;

PEP8 represents a CTL epitope peptide represented by SEQ ID NO: 8;

PEP9 represents a CTL epitope peptide represented by SEQ ID NO: 9;

PEP10 represents a CTL epitope peptide represented by SEQ ID NO: 10;

PEP13 represents a CTL epitope peptide represented by SEQ ID NO: 13;

PEP15 represents a CTL epitope peptide represented by SEQ ID NO: 15;

PEP18 represents a CTL epitope peptide represented by SEQ ID NO: 18,

wherein the concentration of 3 types of peptides having 4 linked CTL epitopes in the aqueous phase in total is 3 to 27 mg/ml.

[2] The water-in-oil emulsion formulation according to claim 1, which contains 3 types of peptides having 4 linked CTL epitopes and an oil adjuvant.

[3] The water-in-oil emulsion formulation according to claim 1 or 2, which is obtained by mixing an aqueous phase comprising peptides having 4 linked CTL epitopes and an oil phase comprising an oil adjuvant and emulsifying the mixture~~, wherein the aqueous phase comprising peptides having 4 linked CTL epitopes is a solution comprising 3 types of peptides having 4 linked CTL epitopes: a peptide represented by Formula (I); a peptide represented by Formula (II); and a peptide represented by Formula (III):~~

~~A (L) B (L) C (L) PEP2 (I);~~

~~D (L) E (L) F (L) PEP4 (II); and~~

~~G (L) H (L) I (L) PEP10 (III),~~

~~in Formulae (I), (II), and (III),~~

~~(L) represents a linker;~~

~~PEP2 represents a CTL epitope peptide represented by SEQ ID NO: 2;~~

~~PEP4 represents a CTL epitope peptide represented by SEQ ID NO: 4;~~

~~PEP10 represents a CTL epitope peptide represented by SEQ ID NO: 10;~~

~~A, B, C, D, E, F, G, H, and I are each independently selected from the group consisting of a CTL epitope peptide represented by SEQ ID NO: 1 (PEP1), a CTL epitope peptide represented by SEQ ID NO: 5 (PEP5), a CTL epitope peptide represented by SEQ ID NO: 6 (PEP6), a CTL epitope peptide represented by SEQ ID NO: 7 (PEP7), a CTL epitope peptide represented by SEQ ID NO: 8 (PEP8), a CTL epitope peptide represented by SEQ ID NO: 9 (PEP9), a CTL epitope peptide represented by SEQ ID NO: 13 (PEP13), a CTL epitope peptide represented by SEQ ID NO: 15 (PEP15), and a CTL epitope peptide represented by SEQ ID NO: 18 (PEP18), except for a combination of A representing PEP7 and B representing PEP8; and~~

~~the peptides having 4 linked CTL epitopes represented by Formulae (I), (II), and (III) may each have a peptide sequence consisting of hydrophilic amino acids~~.

~~[4] The emulsion formulation according to claim 3, wherein, in Formulae (I), (II), and~~

~~(III), (L) represents a linker; A, B, and C are each independently selected from the group consisting of PEP7, PEP8, and PEP13, except for a combination of A representing PEP7 and B representing PEP8; D, E, and F are each independently selected from the group consisting of PEP5, PEP6, and PEP9; and G, H, and I are each independently selected from the group consisting of PEP1, PEP15, and PEP18, and the peptide represented by Formula (I) may have a peptide sequence consisting of hydrophilic amino acids.~~

[~~5~~4] The emulsion formulation according to any one of claims 1 to ~~claim~~ 3 ~~or 4~~, wherein the peptide represented by Formula (I) is selected from the group consisting of:

·PEP7-(L)-PEP13-(L)-PEP8-(L)-PEP2;

·PEP8-(L)-PEP7-(L)-PEP13-(L)-PEP2;

·PEP8-(L)-PEP13-(L)-PEP7-(L)-PEP2;

·PEP13-(L)-PEP7-(L)-PEP8-(L)-PEP2; and

·PEP13-(L)-PEP8-(L)-PEP7-(L)-PEP2,

the peptide represented by Formula (II) is selected from the group consisting of:

·PEP5-(L)-PEP6-(L)-PEP9-(L)-PEP4;

·PEP5-(L)-PEP9-(L)-PEP6-(L)-PEP4;

·PEP6-(L)-PEP5-(L)-PEP9-(L)-PEP4;

·PEP6-(L)-PEP9-(L)-PEP5-(L)-PEP4;

·PEP9-(L)-PEP5-(L)-PEP6-(L)-PEP4; and

·PEP9-(L)-PEP6-(L)-PEP5-(L)-PEP4, and

the peptide represented by Formula (III) is selected from the group consisting of:

·PEP1-(L)-PEP15-(L)-PEP18-(L)-PEP10;

·PEP1-(L)-PEP18-(L)-PEP15-(L)-PEP10;

·PEP15-(L)-PEP1-(L)-PEP18-(L)-PEP10;

·PEP15-(L)-PEP18-(L)-PEP1-(L)-PEP10;

·PEP18-(L)-PEP1-(L)-PEP15-(L)-PEP10; and

·PEP18-(L)-PEP15-(L)-PEP1-(L)-PEP10, and

the peptide represented by Formula (I) ~~may have a peptide sequence consisting of hydrophilic amino acids~~ has an arginine tetramer at the N terminus.

~~[6] The emulsion formulation according to any one of claims 3 to 5, wherein, in~~

~~Formulae (I), (II), and (III), (L) represents an arginine dimer; and a peptide sequence consisting of hydrophilic amino acids is an arginine tetramer.~~

[~~7~~5] The emulsion formulation according to ~~any one of claims 3 to 6~~claim 4, wherein the 3 types of peptides having 4 linked CTL epitopes represented by Formulae (I), (II), and (III) are RRRR-PEP7-RR-PEP13-RR-PEP8-RR-PEP2 (SEQ ID NO: 19), PEP5-RR-PEP9-RR-PEP6-RR-PEP4 (SEQ ID NO: 25), and PEP15-RR-PEP18-RR-PEP1-RR-PEP10 (SEQ ID NO: 33).

[~~8~~6] A method for preparing the emulsion formulation according to any one of claims 1 to ~~7~~ 5 comprising the steps (i) and (ii):

(i) a step of injecting a part of an aqueous phase comprising peptides having 4 linked CTL epitopes into an oil phase comprising an oil adjuvant to prepare a mixture; and

(ii) a step of allowing the mixture to reciprocate between syringes connected via a syringe connector,

wherein the step (i) and the step (ii) are repeated to obtain an emulsion formulation comprising the total amount of the oil phase and the aqueous phase, and wherein the amount of a part of the aqueous phase is 1/10 to 1/2 fold (v/v) relative to the amount of the oil phase comprising an oil adjuvant.

~~[9] The method according to claim 8, wherein the amount of a part of the aqueous phase is 1/10 to 1/2 fold (v/v) relative to the amount of the oil phase comprising an oil adjuvant.~~

[~~10~~7] ~~An~~ Use of an apparatus ~~used~~ for preparing a water-in-oil emulsion formulation according to any one of claims 1 to 5~~containing peptides, which~~, wherein the apparatus is composed of:

a second syringe 2;

a syringe connector 5 connected to the second syringe 2;

a three-way stopcock 4 connected to the syringe connector 5; and

a first syringe 1 and a third syringe 3 further connected to the three-way stopcock 4,

wherein the apparatus enables production of a water-in-oil emulsion formulation by adding an aqueous phase filled in the third syringe 3 to the oil phase filled in the first syringe 1 or the second syringe 2 and allowing the resulting mixture to reciprocate between the first syringe 1 and the second syringe 2 via the syringe connector 5.

[~~11~~8] The ~~apparatus~~ use according to claim ~~10~~7, wherein the volumes of the first, the second, and the third syringes are each 3 ml or less.

[~~12~~9] The ~~apparatus~~ use according to claim ~~10~~ 7 or ~~11~~8, wherein an inner diameter of the syringe connector 5 through which the mixture passes is within a range from 0.8 to 1.7 mm.

[~~13~~10] ~~A kit used~~Use of a kit for preparing an w/o emulsion formulation according to any one of claims 1-5, ~~containing peptides having 4 linked CTL epitopes, which~~ wherein the kit comprises peptides having 4 linked CTL epitopes each comprising ~~4~~ CTL epitope peptides linked via linkers, an oil adjuvant, and the apparatus according to any one of claims ~~10~~ 7 to ~~12~~9.

~~[14] The water-in-oil (w/o) emulsion formulation according to any one of claims 1 to 7 for use in the treatment of tumor.~~

[~~15~~11] Use of a solution of peptides having 4 linked CTL epitopes in the manufacture of the water-in-oil (w/o) emulsion formulation according to any one of claims 1 to ~~7~~5.

~~[16] The apparatus according to any one of claims 10 to 12 or the kit according to claim 13, which is used for preparing the water-in-oil (w/o) emulsion formulation for the treatment of tumor at the time of use.~~

~~[17] The apparatus according to any one of claims 10 to 12 or the kit according to claim 13, wherein the water-in-oil (w/o) emulsion formulation is the formulation according to any one of claims 1 to 7.~~