Current biopharmaceutical practice is mainly concerned with the hydrolytic degradation of PS20 in biopharmaceutical formulations due to the presence of enzymes (36,37).

Different strategies for mitigating PS degradation have been investigated. For example, the effect of different excipients such as EDTA, methionine, citrate, or butylhydroxytoluene (BHT) on oxidative degradation of PS has been evaluated, however divergent conclusions have been reported and even dual effects have been observed (24,38–40).

Doyle *et al.* (38) describe that PS80 remains stable for several days in high buffer concentrations of 10 mM histidine buffer or in 10 mM citrate buffer. Doyle *et al.* also report that histidine buffer containing citrate prevents oxidation of PS80 in a short-time study for up to 7 days.

Gopalrathnam *et al.* (47) describe studies of degradation of PS80 and PS20 at high concentrations of histidine and/or citrate, and specifically in histidine buffer and/or 10 mM citrate buffer.

**Summary**

The present disclosure provides an aqueous formulation comprising polysorbate, citrate and sodium chloride. The present disclosure also provides an aqueous formulation consisting essentially of polysorbate, citrate, sodium chloride and water. In some examples, the polysorbate is polysorbate polysorbate 80. Preferably, the polysorbate is polysorbate 20.

In one aspect, the present disclosure provides an aqueous formulation comprising polysorbate 20, citrate and sodium chloride, wherein the concentration of citrate in the aqueous formulation is from 250 µM to 1 mM and wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from 1.5 to 7.

In particular, the present disclosure provides an aqueous formulation comprising polysorbate 20, citrate and sodium chloride. The present disclosure also provides an aqueous formulation consisting essentially of polysorbate 20, citrate, sodium chloride and water.

In some examples of the present disclosure, the concentration of citrate in the aqueous formulation is less than 10 mM, preferably less than 5 mM, more preferably less than 3 mM. Higher concentrations of citrate may, e.g., result in formation of protein particles, if a protein or peptide is added, and/or promote gelation. Accordingly, it is preferred that the concentration of citrate in the aqueous formulation is from about 50 µM to about 2.5 mM, more preferably from about 250 µM to about 1 mM. Still more preferably, the concentration of citrate in the aqueous formulation is from about 0.25 mM to about 0.75 mM. Most preferably, the concentration of citrate is about 0.5 mM.

In some examples, the concentration of polysorbate (*e.g* polysorbate 20 or polysorbate 80) in the aqueous formulation is about 0.02% (w/v).

The present disclosure also provides the use of citrate at a final molar ratio of citrate to polysorbate of about 1.5 to about 7, to prevent degradation of polysorbate in an aqueous formulation. Also provided is a method for preventing degradation of polysorbate in an aqueous formulation, comprising adding citrate at a final molar ratio of citrate to polysorbate of about 1.5 to about 7. In some examples, the polysorbate is polysorbate 80. Preferably, the polysorbate is polysorbate 20.

In the aqueous formulation, method or use of the disclosure, the molar ratio (or final molar ratio) of citrate to polysorbate in the aqueous formulation is from about 1.5 to about 7, and preferably, from about 3 to about 6. More preferably, the molar ratio (or final molar ratio) of citrate to polysorbate is about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, or about 3.9.

The present disclosure also provides the use of citrate at a final molar ratio of citrate to polysorbate 20 of about 0.31 to about 15.4, preferably of about 1.5 to about 7, to prevent degradation of polysorbate 20 in an aqueous formulation. Also provided is a method for preventing degradation of polysorbate 20 in an aqueous formulation, comprising adding citrate at a final molar ratio of citrate to polysorbate of about 1.5 to about 7.

In another aspect, the present disclosure provides a method for preventing degradation of polysorbate 20 in the aqueous formulation as described herein, the method comprising adding citrate at a final molar ratio of citrate to polysorbate 20 of 1.5 to 7.

In another aspect, the present disclosure provides use of citrate at a final molar ratio of citrate to polysorbate 20 of 1.5 to 7 to prevent degradation of polysorbate 20 in the aqueous formulation as described herein.

In the aqueous formulation, method or use of the disclosure, the molar ratio (or final molar ratio) of citrate to polysorbate 20 in the aqueous formulation is preferably from about 1.5 to about 7. More preferably, the molar ratio (or final molar ratio) of citrate to polysorbate 20 is from about 3 to about 6. In certain examples, the molar ratio (or final molar ratio) of citrate to polysorbate 20 is about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, or about 3.9.

In the aqueous formulation, method or use of the disclosure, the molar ratio (or final molar ratio) of citrate to polysorbate 80 in the aqueous formulation is preferably from about 1.6 to about 7. More preferably, the molar ratio (or final molar ratio) of citrate to polysorbate 80 is from about 3.2 to about 6.5. In certain examples, the molar ratio (or final molar ratio) of citrate to polysorbate 80 is about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, or about 3.9.

In some examples of the aqueous formulations, methods or uses of the disclosure, the aqueous formulation comprises sodium chloride at a concentration of about 0.9 % (w/v). In some examples, the aqueous formulation does not comprise histidine and/or further comprises a metal ion. In some examples, the concentration of sodium chloride in the aqueous formulation is about 0.9 % (w/v), and the molar ratio of citrate to polysorbate (*e.g.* polysorbate 20, polysorbate 80 *etc*.) in the aqueous formulation is from about 3 to about 6. In some examples, the aqueous formulation consists essentially of polysorbate 20, citrate, water and sodium chloride. In some examples, the aqueous formulation further comprises a pharmaceutically-acceptable carrier, diluent, excipient or adjuvant. In some examples, the aqueous formulation further comprises a peptide/polypeptide or complex thereof for use in therapy.

In some examples of the aqueous formulations, methods or uses of the disclosure, the aqueous formulation consists essentially of:

- about 0.02% (w/v) polysorbate,
- about 0.9 % (w/v) sodium chloride,
- water,
- 0.25 mM to 1 mM citrate, and

- optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

Preferably, said aqueous formulation consists essentially of:

- about 0.02% (w/v) polysorbate,
- about 0.9 % (w/v) sodium chloride,
- water,
- about 0.5 mM citrate, and
- optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

In some examples of the aqueous formulations, methods or uses of the disclosure, the aqueous formulation comprises polysorbate 80 and consists essentially of:

- about 0.02% (w/v) polysorbate 80,
- about 0.9 % (w/v) sodium chloride,
- water,
- 0.25 mM to 1 mM citrate, and
- optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

Preferably, said aqueous formulation comprising polysorbate 80 consists essentially of:

- about 0.02% (w/v) polysorbate 80,
- about 0.9 % (w/v) sodium chloride,
- water,
- about 0.5 mM citrate, and
- optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

In other examples of the aqueous formulations, methods or uses of the disclosure, the aqueous formulation comprises polysorbate 20 and consists essentially of:

- about 0.02% (w/v) polysorbate 20 (PS20),
- about 0.9 % (w/v) sodium chloride,
- water,
- 0.25 mM to 1 mM citrate, and
- optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

Preferably, said aqueous formulation comprising polysorbate 20 consists essentially of:

- about 0.02% (w/v) polysorbate 20 (PS20),
- about 0.9 % (w/v) sodium chloride,
- water,
- about 0.5 mM citrate, and
- optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

In some examples, the aqueous formulation is suitable for intravenous, parenteral, systemic, intracavitary, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, subconjunctival, intravitreal, intratumoral, subcutaneous, intradermal, oral or transdermal administration.

In another aspect, the present disclosure also provides the use of the aqueous formulation as described herein as a dilution medium for a composition.

The present disclosure also provides a method for preparing a composition, the method comprising contacting a peptide/polypeptide or complex thereof for use in therapy, prophylaxis and/or diagnosis with an aqueous formulation according to the present disclosure. In some examples, the method is a method for preparing a pharmaceutical composition for administration.

In another aspect, the present disclosure provides a method for preparing a composition, the method comprising contacting a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis with an aqueous formulation as described herein.

In some examples, the peptide/polypeptide or complex thereof for use in therapy, prophylaxis and/or diagnosis is selected from: an antigen-binding peptide/polypeptide, an antigen-binding peptide/polypeptide complex, an antibody or an antigen-binding fragment thereof, an Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein, an enzyme, a growth factor, a hormone, an interferon, an interleukin, and a thrombolytic.

The present disclosure also provides a composition obtained or obtainable by any of the methods of the present disclosure.

**Description**

The present disclosure relates to aqueous formulations comprising polysorbate and citrate, in particular to aqueous formulations comprising polysorbate 20 and citrate.

In the experimental examples of the present disclosure, the inventors demonstrate that such formulations stabilise (*i.e.* prevent degradation of) polysorbate, such as polysorbate 20 and polysorbate 80.

The use of a molar ratio of citrate to polysorbate (*e.g.* polysorbate 80 or polysorbate 20 *etc.*) which lies within an optimal range of about 1.5 to about 7, preferably about 1.6 to about 7, and more preferably about 3 to about 6, is demonstrated to prevent degradation of polysorbate. A lower ratio (*e.g.* below about 1.6) or a higher ratio (*e.g.* about 16) is demonstrated to be less effective in preventing degradation of the corresponding polysorbate in the aqueous formulation.

In particular, the use of a molar ratio of citrate to polysorbate 20 which lies within an optimal range of about 1.5 to about 7, preferably about 3 to about 6, is demonstrated to prevent degradation of polysorbate 20. A lower ratio (*e.g.* below about 1.5) or a higher ratio (*e.g.* about 15) is demonstrated to be less effective in preventing degradation of PS20 in the aqueous formulation.

**Claims:**

1. An aqueous formulation comprising polysorbate 20, citrate and sodium chloride, wherein the concentration of citrate in the aqueous formulation is from 250 µM to 1 mM and wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from 1.5 to 7.

2. The aqueous formulation of claim 1, wherein the concentration of polysorbate 20 in the aqueous formulation is 0.02% (w/v) +/- 10%.

3. The aqueous formulation of claim 1 or 2, wherein the concentration of sodium chloride in the aqueous formulation is 0.9 % (w/v) +/- 10%.

4. The aqueous formulation of any one of claims 1 to 3, wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from 3 to 6.

5. The aqueous formulation of any one of claims 1 to 4, wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is 3 +/- 10%, 3.1 +/- 10%, 3.2 +/- 10%, 3.3 +/- 10%, 3.4 +/- 10%, 3.5 +/- 10%, 3.6 +/- 10%, 3.7 +/- 10%, 3.8 +/- 10%, or 3.9 +/- 10%.

6. The aqueous formulation of any one of claims 1 to 5, wherein the aqueous formulation does not comprise histidine.

7. The aqueous formulation according to any one of claims 1 to 6, wherein the aqueous formulation consists essentially of polysorbate 20, citrate, water and sodium chloride.

8. The aqueous formulation according to any one of claims 1 to 7, wherein the aqueous formulation consists essentially of:

- 0.02% (w/v) +/- 10% polysorbate 20 (PS20),
- 0.9 % (w/v) +/- 10% sodium chloride,
- water, and
- 0.5 mM +/- 10% citrate.

9. The aqueous formulation of any one of claims 1 to 8, wherein the aqueous formulation is suitable for intravenous, parenteral, systemic, intracavitary, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, subconjunctival, intravitreal, intratumoral, subcutaneous, intradermal, oral or transdermal administration; and/or

wherein the aqueous formulation further comprises a pharmaceutically-acceptable carrier, excipient or adjuvant.

10. The aqueous formulation of any one of claims 1 to 6 and 9, further comprising a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

11. The aqueous formulation of claim 10, wherein the peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis is selected from: an antigen-binding peptide/polypeptide, an antigen-binding peptide/polypeptide complex, an antibody or an antigen-binding fragment thereof, an Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein, an enzyme, a growth factor, a hormone, an interferon, an interleukin, and a thrombolytic.

12. A method for preventing degradation of polysorbate 20 in the aqueous formulation according to any one of claims 1 to 11, the method comprising adding citrate at a final molar ratio of citrate to polysorbate 20 of 1.5 to 7.

13. Use of citrate at a final molar ratio of citrate to polysorbate 20 of 1.5 to 7 to prevent degradation of polysorbate 20 in the aqueous formulation according to any one of claims 1 to 11.

14. Use of the aqueous formulation of any one of claims 1 to 11 as a dilution medium for a composition.

15. The use of claim 14, wherein the composition comprises a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis,

optionally wherein the peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis is selected from: an antigen-binding peptide/polypeptide, an antigen-binding peptide/polypeptide complex, an antibody or an antigen-binding fragment thereof, an Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein, an enzyme, a growth factor, a hormone, an interferon, an interleukin, and a thrombolytic.

16. A method for preparing a composition, the method comprising contacting a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis with an aqueous formulation of any one of claims 1 to 9.