

Formulations Comprising Polysorbate

This application claims priority from EP 23163573.1 filed 22 March 2023, the contents and elements of which are herein incorporated by reference for all purposes.

Technical Field

The present disclosure relates to pharmaceutical science, more specifically biopharmaceutical formulation technology.

5 Background

The development of biopharmaceutical products necessitates the evaluation of conditions and factors that can potentially impact product quality. Critical factors affecting protein stability are for instance mechanical stress, temperature, light exposure, freeze-thaw cycles, raw material impurities, as well as leaching from primary packaging (1–6). Based on their amphiphilic nature and physico-chemical properties, proteins such as monoclonal antibodies (mAb) tend to adsorb to hydrophobic interfaces (e.g., solution/air interface, plastic polymers, glass, stainless steel), which may lead to partial unfolding, followed by aggregation and potentially particle formation (6,7).

The common goal of biopharmaceutical formulation development is to minimize these risks to ensure safety and efficacy of the final drug product. Therefore, it is important to focus on chemical, colloidal and conformational protein stability, during the pharmaceutical development. The goal of pharmaceutical development is to provide an appropriate, patient friendly formulation (e.g., buffer system, tonicity agent, surfactant) to stabilize the protein (such as mAb). In addition, clinical handling and in-use stability studies are performed for e.g. testing the drug product with clinical dilution and infusion media. As patient safety is of highest priority, such tests investigate protein stability and the potential formation of protein particles. Especially for medications delivered through an infusion, the dilution of the drug using an infusion bag may reduce the stability of the drug in the diluted condition (8–10).

Current commercial dilution media used in the clinic include normal saline (0.9 % (w/v) sodium chloride), half normal saline solution, lactated Ringer's and 5 % (w/v) dextrose solution. Various research articles have reported the incompatibility of proteins with such dilution media and infusion components (11–15). The phenomenon of particle formation has been described in the literature, mainly in relation to high antibody (e.g. monoclonal antibody) dilution (9,14,16–19). Some reports have evaluated the use of in-line filters to remove particles. Particle removal, however, needs to be verified for each in-line filter and product, because the use of in-line filters could potentially affect the physicochemical stability of the protein as well as drug recovery (10,11,20). In specific cases, the prescribing information for dilution of certain biologics, such as Herceptin® and Avastin® require dilution of the drug using normal saline and specifically prohibits the use of dextrose ('Do not use dextrose (5%) solution') (15). The dilution in dextrose-containing solutions bears, in addition to the risk of affecting the physicochemical stability of the protein, the risk for glycation between the sugar and the pharmaceutical-active protein, which result in a loss of binding activity and thereby reduced efficacy (21).

As a potential solution, dedicated diluent solutions with the same excipient composition as the drug product can be used. However, the use of specific dilution media for the drug products reduces the flexibility in clinic, requires an additional manufacturing process and related stability activities and
5 duplicates the supply chain activities. To reduce the development efforts for a suitable diluent, a generic diluent that would allow the stabilization of most drug products in the diluted application would be beneficial. Such a diluent would be beneficial for use in preparing infusions for use in the clinic. For example, during dose-escalation approaches in phase I cancer clinical trials, highly diluted infusions are used, such that the drug product need to be diluted to a factor of e.g. 10^3 to 10^8 compared to the drug
10 product concentration (22).

Polysorbates (PS) are amphipathic, non-ionic surfactants that are widely used in therapeutic protein formulations due to their low toxicity, high biocompatibility, and their high stabilizing ability (3). Due to its amphiphilic character, PS saturates hydrophobic interfaces and thereby leads to enhancement of physical
15 and colloidal stability of proteins (6,23).

Polysorbate (PS) is composed of sorbitan/isosorbide linked to polyoxyethylene chains (POE), via esterified fatty acids. The heterogeneity of PS is based on the degree of ethoxylation and esterification as well as on the mixture of fatty acids used in the preparation of PS. Polysorbate 20 (PS20) comprises
20 mainly esterified lauric acid (40-60 %), whereas polysorbate 80 (PS80) mainly comprises esterified oleic acid (>58 %) (17,24). The exact composition of compendial grade PS20 / PS80 is defined in the pharmacopoeias (Ph. Eur., USP, JP, BP, or ChP). PS20 and PS80 are also known as Tween® 20 and Tween® 80, respectively.

25 Figure 1 shows an idealized chemical structure of PS20.

The stability of PS in aqueous formulations has some liabilities. Two main degradation pathways are currently considered: the hydrolytic pathway (chemical and enzymatic hydrolysis), targeting the fatty acid ester bond, and the oxidative pathway, targeting the hydrocarbon chain double bond of unsaturated fatty
30 acids and/or the polyoxyethylene (POE) chains (25,26) (Figure 1). Both degradation pathways are of potential risks for the integrity and shelf-life of active pharmaceutical ingredients (27,28), as well as for clinically-relevant diluents containing PS.

While host cell proteins are identified as a major root cause for hydrolytic enzymatic degradation (29,30)
35 several factors can promote oxidative degradation of PS. In principle, the presence of reactive oxygen species (ROS) is a prerequisite (31,32). Origin of ROS like residual hydrogen peroxide (H_2O_2) or organic peroxides, may be the raw materials or the manufacturing/filling process, as H_2O_2 is used as a decontamination agent in manufacturing (33,34). These factors lead to degradation of PS and, consequently, destabilisation of the drug product contained in the formulation since the surfactant
40 functionality of PS is impaired (35).

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

5 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).

10 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.

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Summary

In a first aspect, 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 embodiments, the polysorbate is polysorbate 20 polysorbate 80. Preferably, the polysorbate is polysorbate 20.

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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.

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In some embodiments of the various aspects 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 μ M to about 2.5 mM, more preferably from about 250 μ M 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.

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

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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 embodiments, the polysorbate is polysorbate 80. Preferably, the polysorbate is polysorbate 20.

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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 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 embodiments, 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 embodiments, 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 embodiments 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 embodiments, the aqueous formulation does not comprise histidine and/or further comprises a metal ion. In some embodiments, 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 embodiments, the aqueous formulation consists essentially of polysorbate 20, citrate, water and sodium chloride. In some embodiments, the aqueous formulation further comprises a pharmaceutically-acceptable carrier, diluent, excipient or adjuvant. In some embodiments, the aqueous formulation further comprises a peptide/polypeptide or complex thereof for use in therapy.

In some embodiments 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,
- 5 - 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.

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In some embodiments 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,
- 15 - 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:

- 20 - 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,
- 25 - optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

In other embodiments 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),
- 30 - 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.

35 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
- 40 - optionally, a pharmaceutically-acceptable carrier, excipient or adjuvant,
- optionally, a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

In some embodiments, 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.

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The present disclosure also provides the use of the aqueous formulation of any of the aspects 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 embodiments, the method is a method for preparing a pharmaceutical composition for administration.

In some embodiments, 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.

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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.

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Polysorbate

Polysorbate (PS) is a class of amphipathic, non-ionic surfactants. The term 'polysorbate' refers to a surfactant which is composed of sorbitan/isosorbide linked to polyoxyethylene chains (POE), via esterified fatty acids. Exemplary polysorbates include, e.g., polysorbate 20, 40, 60, 65 and 80. Polysorbate 20, 40, 60, and 80 utilise lauric, palmitic, stearic and oleic acid, respectively, for the fatty acid portion of the molecule.

Polysorbate 20 (PS20) is polyoxyethylene sorbitan monolaurate. Polysorbate 80 (PS80) is polyoxyethylene sorbitan monooleate. Polysorbate 20 (PS20) comprises mainly esterified lauric acid (40-60 %), whereas polysorbate 80 (PS80) mainly comprises esterified oleic acid (>58 %) (17,24). The exact composition of compendial grade PS20 / PS80 is defined in the pharmacopoeias (Ph. Eur., USP, JP, BP, or ChP). Polysorbate 40 is polyoxyethylene sorbitan monopalmitate. Polysorbate 60 is polyoxyethylene sorbitan monostearate, whereas polysorbate 65 is a polyoxyethylene sorbitan tristearate.

In some embodiments, polysorbate is or comprises polysorbate 20, polysorbate 40, polysorbate 60 and/or polysorbate 80.

In some embodiments of aspects of the disclosure, polysorbate is polysorbate 20. In some embodiments, polysorbate is polysorbate 80. In some embodiments, polysorbate is polysorbate 60. In some embodiments, polysorbate is polysorbate 40. Preferably, in the present disclosure, polysorbate is polysorbate 20.

In some embodiments, the concentration of polysorbate (e.g. polysorbate 80) in an aqueous formulation according to the present disclosure is from about 0.001 % (w/v) (= 0.01 g/L) to about 0.06 % (w/v) (= 0.6 g/L), e.g. one of: from about 0.001 % (w/v) to about 0.01 % (w/v), from about 0.005 % to about 0.01 % (w/v), from about 0.005 % (w/v) to about 0.015 %, from about 0.01 % (w/v) to about 0.02 %, from about 0.015 % (w/v) to about 0.02 % (w/v), from about 0.015 % (w/v) to about 0.025 % (w/v), from about 0.02 % (w/v) to about 0.03 % (w/v), from about 0.025 % (w/v) to about 0.03 %, from about 0.025 % (w/v) to about 0.035 % (w/v), from about 0.03 % (w/v) to about 0.04 % (w/v), from about 0.035 % (w/v) to about 0.04 % (w/v), from about 0.035 % (w/v) to about 0.045 % (w/v), from about 0.04 % (w/v) to about 0.05 % (w/v), from about 0.045 % (w/v) to about 0.05 % (w/v), from about 0.045 % (w/v) to about 0.055 % (w/v), from about 0.05 % (w/v) to about 0.06 % (w/v), and from about 0.055 % (w/v) to about 0.06 % (w/v). In some embodiments, the concentration is from about 0.01 % (w/v) to about 0.06 % (w/v), e.g. one of: about 0.02 to about 0.06 % (w/v), from about 0.03 % (w/v) to about 0.06 % (w/v), from about 0.04 % (w/v) to about 0.06 % (w/v), and from about 0.05 % (w/v) to about 0.06 % (w/v).

It is preferred that the concentration of polysorbate (e.g. polysorbate 80) in an aqueous formulation according to the present disclosure is about 0.01 % (w/v) to about 0.06 % (w/v), preferably about 0.015 % (w/v) to about 0.04 % (w/v), 0.02 % (w/v) to about 0.04 % (w/v), more preferably about 0.015 % (w/v) to about 0.03 % (w/v), 0.02 % (w/v) to about 0.03 % (w/v), even more preferably about 0.02 % (w/v). A concentration of 0.02 % (w/v) is equivalent to 0.2 mg·ml⁻¹.

In other embodiments, the concentration of polysorbate (e.g. polysorbate 80) is about 0.01 % (w/v). It will be appreciated that higher concentrations of polysorbate may be used. Accordingly, in some embodiments, the concentration of polysorbate (e.g. polysorbate 80) in the aqueous formulation is about
5 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v) or about 0.06 % (w/v).

In some embodiments, the concentration of polysorbate (e.g. polysorbate 80) in an aqueous formulation according to the present disclosure is from about 8 μ M to about 0.46 mM, e.g. one of: from about 8 μ M to
10 about 0.08 mM, from about 0.04 mM to about 0.08 mM, from about 0.04 mM to about 0.11 mM, from about 0.08 mM to about 0.14 mM, from about 0.10 mM to about 0.15 mM, from about 0.08 mM to about 0.19 mM, from about 0.17 mM to about 0.23 mM, from about 0.19 mM to about 0.23 mM, from about 0.19 mM to about 0.23 mM, from about 0.23 mM to about 0.31 mM, from about 0.27 mM to about 0.31 mM, from about 0.27 mM to about 0.34 mM, from about 0.31 mM to about 0.38 mM, from about 0.34 mM to
15 about 0.38 mM, from about 0.34 mM to about 0.42 mM, from about 0.38 mM to about 0.46 mM, and from about 0.42 mM to about 0.46 mM.

It is preferred that the concentration of polysorbate (e.g. polysorbate 80) in an aqueous formulation according to the present disclosure is about 80 μ M to about 0.49 mM, preferably about 0.12 mM to about
20 0.33 mM, 0.17 mM to about 0.33 mM, more preferably about 0.12 mM to about 0.25 mM, 0.15 mM to about 0.25 mM, even more preferably about 0.15 mM. A concentration of 0.15 mM of e.g. PS80, is equivalent to 0.2 mg·mL⁻¹.

In other embodiments, the concentration of polysorbate (e.g. polysorbate 80) is about 0.008 mM. It will be
25 appreciated that higher concentrations of polysorbate may be used. Accordingly, in some embodiments, the concentration of polysorbate in the aqueous formulation is about 0.008 mM, about 0.15 mM, about 0.23 mM, about 0.31 mM, about 0.38 mM or about 0.46 mM.

In some embodiments of various aspects of the disclosure, polysorbate is not polysorbate 80 (PS80).
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Preferably, in the present disclosure, the polysorbate is polysorbate 20. In some embodiments, the concentration of polysorbate 20 in an aqueous formulation according to the present disclosure is from about 0.001 % (w/v) (= 0.01 g/L) to about 0.06 % (w/v) (= 0.6 g/L), e.g. one of: from about 0.001 % (w/v) to about 0.01 % (w/v), from about 0.005 % to about 0.01 % (w/v), from about 0.005 % (w/v) to about
35 0.015 %, from about 0.01 % (w/v) to about 0.02 %, from about 0.015 % (w/v) to about 0.02 % (w/v), from about 0.015 % (w/v) to about 0.025 % (w/v), from about 0.02 % (w/v) to about 0.03 % (w/v), from about 0.025 % (w/v) to about 0.03 %, from about 0.025 % (w/v) to about 0.035 % (w/v), from about 0.03 % (w/v) to about 0.04 % (w/v), from about 0.035 % (w/v) to about 0.04 % (w/v), from about 0.035 % (w/v) to about 0.045 % (w/v), from about 0.04 % (w/v) to about 0.05 % (w/v), from about 0.045 % (w/v) to about 0.05 %
40 (w/v), from about 0.045 % (w/v) to about 0.055 % (w/v), from about 0.05 % (w/v) to about 0.06 % (w/v),

and from about 0.055 % (w/v) to about 0.06 % (w/v). In some embodiments, the concentration is from about 0.01 % (w/v) to about 0.06 % (w/v), e.g. one of: about 0.02 to about 0.06 % (w/v), from about 0.03 % (w/v) to about 0.06 % (w/v), from about 0.04 % (w/v) to about 0.06 % (w/v), and from about 0.05 % (w/v) to about 0.06 % (w/v).

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It is preferred that the concentration of polysorbate 20 in an aqueous formulation according to the present disclosure is about 0.01 % (w/v) to about 0.06 % (w/v), preferably about 0.015 % (w/v) to about 0.04 % (w/v), 0.02 % (w/v) to about 0.04 % (w/v), more preferably about 0.015 % (w/v) to about 0.03 % (w/v), 0.02 % (w/v) to about 0.03 % (w/v), even more preferably about 0.02 % (w/v). A concentration of 0.02 % (w/v) is equivalent to 0.2 mg·ml⁻¹.

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In other embodiments, the concentration of polysorbate 20 is about 0.01 % (w/v). It will be appreciated that higher concentrations of polysorbate 20 may be used. Accordingly, in some embodiments, the concentration of polysorbate 20 in the aqueous formulation is about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v) or about 0.06 % (w/v).

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The concentration of a polysorbate in an aqueous formulation according to the present disclosure may be provided in molar concentration (mol/L or M). Accordingly, the molecular weight of the polysorbate may be used to convert the (w/v) concentration to molar concentration. The molecular weight of PS20 used for the purposes of such calculations is 1227 g/mol. The molecular weight of PS80 used for the purposes of such calculations, e.g., for comparison, is 1310 g/mol.

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In some embodiments, the concentration of polysorbate 20 in an aqueous formulation according to the present disclosure is from about 8 μM to about 0.48 mM, e.g. one of: from about 8 μM to about 0.08 mM, from about 0.04 mM to about 0.08 mM, from about 0.04 mM to about 0.12 mM, from about 0.08 mM to about 0.16 mM, from about 0.12 mM to about 0.16 mM, from about 0.08 mM to about 0.2 mM, from about 0.18 mM to about 0.24 mM, from about 0.2 mM to about 0.24 mM, from about 0.2 mM to about 0.28 mM, from about 0.24 mM to about 0.32 mM, from about 0.28 mM to about 0.32 mM, from about 0.28 mM to about 0.36 mM, from about 0.32 mM to about 0.41 mM, from about 0.36 mM to about 0.41 mM, from about 0.36 mM to about 0.45 mM, from about 0.41 mM to about 0.48 mM, and from about 0.45 mM to about 0.48 mM.

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It is preferred that the concentration of polysorbate 20 in an aqueous formulation according to the present disclosure is about 80 μM to about 0.49 mM, preferably about 0.12 mM to about 0.33 mM, 0.17 mM to about 0.33 mM, more preferably about 0.12 mM to about 0.25 mM, 0.17 mM to about 0.25 mM, even more preferably about 0.17 mM. A concentration of 0.17 mM is equivalent to 0.2 mg·ml⁻¹.

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In other embodiments, the concentration of polysorbate 20 is about 80 μM. It will be appreciated that higher concentrations of polysorbate 20 may be used. Accordingly, in some embodiments, the

concentration of polysorbate 20 in the aqueous formulation is about 80 μ M, about 0.17 mM, about 0.25 mM, about 0.33 mM, about 0.41 mM or about 0.49 mM.

Aqueous formulations

5 An 'aqueous formulation' refers to a solution comprising water. In preferred embodiments, an aqueous formulation according to the present disclosure employs water as a solvent. Aqueous formulations according to the present disclosure preferably comprise one or more solute compounds (*i.e.* one or more compounds dissolved in a solvent). In some embodiments, the main solvent for the solute compounds of an aqueous formulation according to the present disclosure is water.

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An aqueous formulation according to the present disclosure may also be employed as a diluent. For example, the aqueous formulations may be used as a diluent for an agent of interest, *e.g.* an agent useful in therapy, prophylaxis and/or diagnosis, or as a diluent for a composition comprising such an agent of interest. The aqueous formulation according to the present disclosure may thus consist of, or consist essentially of, polysorbate 20, citrate, sodium chloride and water.

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The aqueous formulation according to the present disclosure may consist of, or consist essentially of, polysorbate (*e.g.* polysorbate 80), citrate, sodium chloride and water.

20 As used herein, a composition (*e.g.* an aqueous solution) 'consisting essentially of', or that 'consists essentially of' one or more specified constituent(s) lacks further, non-specified constituents at greater than trace amounts. In some embodiments, in particular, in a composition (*e.g.* an aqueous solution) 'consisting essentially of', or that 'consists essentially of' one or more specified constituent(s), any additional, non-specified constituents account for less than 5% (w/v) of the composition, *e.g.* one of ≤ 1 % (w/v), ≤ 0.5 % (w/v), ≤ 0.1 % (w/v), ≤ 0.05 % (w/v) or ≤ 0.01 % (w/v) of the composition. By way of illustration, in an aqueous formulation according to the present disclosure consisting essentially of polysorbate 20, citrate, sodium chloride and water, any constituents of the aqueous formulation other than polysorbate 20, citrate, sodium chloride and water constitute less than 5% (w/v) of the composition, *e.g.* one of ≤ 1 % (w/v), ≤ 0.5 % (w/v), ≤ 0.1 % (w/v), ≤ 0.05 % (w/v) or ≤ 0.01 % (w/v) of the aqueous formulation.

30 In some embodiments, any additional, non-specified constituents are present at less than 5 mM, *e.g.* one of ≤ 1 mM, ≤ 0.5 mM, ≤ 0.1 mM, ≤ 0.05 mM or ≤ 0.01 mM. By way of illustration, in an aqueous formulation according to the present disclosure consisting essentially of polysorbate, citrate, sodium chloride and water, any constituents of the aqueous formulation other than polysorbate, citrate, sodium chloride and water have a concentration of less than 5 mM, *e.g.* one of ≤ 1 mM, ≤ 0.5 mM, ≤ 0.1 mM, ≤ 0.05 mM or ≤ 0.01 mM in the aqueous formulation. It will be appreciated that compositions consisting essentially of one or more specified constituent(s) encompass compositions consisting of only the one or more specified constituent(s). Thus, in some embodiments, a composition that consists essentially of one or more specified constituent(s) may be a composition that consists of those one or more specified constituent(s).

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40 The aqueous formulation comprises polysorbate, *e.g.* polysorbate 20, or polysorbate 80 *etc.*, as described herein. Preferably, the polysorbate comprised in the aqueous formulation is polysorbate 20.

In some embodiments, the aqueous formulation comprises sodium chloride (NaCl). In some embodiments, the aqueous formulation comprises normal saline. Accordingly, the aqueous formulation preferably comprises sodium chloride at a concentration of about 0.9 % (w/v) (= 9 g/L). Accordingly, the aqueous formulation, in some embodiments, is an isotonic solution.

In other embodiments, the aqueous formulation comprises half normal saline, that is the aqueous formulation sodium chloride at a concentration of 0.45 % (w/v). Accordingly, the aqueous formulation, in some embodiments, is a hypotonic solution.

In some embodiments, the concentration of NaCl in an aqueous formulation according to the present disclosure is from about 0.1 % (w/v) (= g/L) to about 1.7 % (w/v) (= 17 g/L), e.g. one of: from about 0.1 % (w/v) to about 1.7 % (w/v), from about 0.2 % (w/v) to about 1.6% (w/v), from about 0.3 % (w/v) to about 1.5% (w/v), from about 0.5 % (w/v) to about 1.4 % (w/v), from about 0.4 % (w/v) to about 1.3% (w/v), from about 0.5 % (w/v) to about 1.2% (w/v), from about 0.6 % (w/v) to about 1.1 % (w/v), from about 0.7 % (w/v) to about 1.0 % (w/v) or from about 0.85 % (w/v) to about 0.95 % (w/v). In some embodiments, the concentration of NaCl in an aqueous formulation according to the present disclosure is about 0.9 % (w/v).

In some embodiments, the aqueous formulation comprises Ringer's lactate solution.

In some embodiments, the aqueous formulation comprises dextrose. In some embodiments, the concentration of dextrose in an aqueous formulation according to the present disclosure is from about 2.5 % (w/v) to about 50 % (w/v), e.g. one of: from about 2.5 % (w/v) to about 5 %, from about 5 % (w/v) to about 10 % (w/v), from about 10 % (w/v) to about 20 % (w/v), from about 20 % (w/v) to about 30 % (w/v), from about 30 % (w/v) to about 40 % (w/v), and from about 40 % (w/v) to about 50 % (w/v). In some embodiments, the concentration of dextrose is about 2.5 % (w/v), about 5 % (w/v), about 10 % (w/v), about 20 % (w/v), about 30 % (w/v) or about 50 % (w/v). In some embodiments, the concentration of dextrose is about 5 % (w/v).

In some embodiments, the aqueous formulation comprises about 0.9 % (w/v) sodium chloride and about 0.2 mg·ml⁻¹ polysorbate (e.g. PS80). Preferably, the polysorbate is polysorbate 20.

Accordingly, in specific embodiments, the aqueous formulation comprises about 0.9 % (w/v) sodium chloride and about 0.2 mg·ml⁻¹ polysorbate 20 (PS20).

The aqueous formulation according to the present disclosure comprises citrate. Citrate is the anion formed by deprotonation one or more carboxy groups of the tricarboxylic acid, citric acid. It can be obtained in solution by addition of citric acid, or a salt of citric acid (e.g. citric acid monohydrate) to water. For example, citrate in an aqueous formulation may be obtained by contacting water with a salt of citric acid, such as sodium citrate dihydrate or trisodium citrate. Suitable salts of citric acid include sodium

citrate dihydrate, disodium citrate, trisodium citrate and combinations thereof. Preferably, sodium citrate (dihydrate) may be used to obtain the aqueous formulation according to the present disclosure.

5 The present disclosure provides a molar ratio of citrate to polysorbate which advantageously helps to prevent degradation of polysorbate (e.g. PS80) in the aqueous formulation. The aqueous formulation of the present disclosure may comprise the following molar ratios. The present disclosure also provides the use of citrate at a final molar ratio of citrate to polysorbate (e.g. PS80) as described herein to prevent degradation of polysorbate (e.g. PS80) in an aqueous formulation as described herein.

10 The present disclosure provides a molar ratio of citrate to polysorbate 20 which advantageously helps to prevent degradation of polysorbate 20 in the aqueous formulation. The aqueous formulation of the present disclosure may comprise the following molar ratios. The present disclosure also provides the use of citrate at a final molar ratio of citrate to polysorbate 20 as described herein to prevent degradation of polysorbate 20 in an aqueous formulation as described herein.

15 In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 1.5 to about 7. In certain embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 1.6 to about 7. In certain embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 3 to about 7. In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 1.5 to about 6. In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 1.6 to about 6. In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 3 to about 6. Preferably, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 3.2 to about 6.5. In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is from about 3 to about 5.

In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) is about 1.6, about 1.7, about 1.8, about 1.9, about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, or about 2.5.

30 In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation 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 some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is about 3.3.

35 In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is about 4, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, or about 4.9.

40 In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is about 5, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, or about 5.9.

In some embodiments, the molar ratio of citrate to polysorbate (e.g. PS80) in the aqueous formulation is about 6, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, or about 6.9.

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In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 1.5 to about 7. In certain embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 3 to about 7. In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 1.5 to about 6. In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 3 to about 6. In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 3 to about 5.

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In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation 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 some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is about 3.

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In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is about 4, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, or about 4.9.

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In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is about 5, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, or about 5.9.

In some embodiments, the molar ratio of citrate to polysorbate 20 in the aqueous formulation is about 6, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, or about 6.9.

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In some embodiments, the aqueous formulation comprises a buffering agent (buffer). A buffering agent (buffer) is used to maintain the pH of the aqueous formulations by the action of its acid-base conjugate components. In some embodiments, the buffering agent is not histidine. In some embodiments, the buffering agent is not phosphate.

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In some embodiments, the aqueous formulation does not comprise histidine. For example, the aqueous formulation does not comprise histidine buffer. In some embodiments, the aqueous formulation does not comprise a histidine buffer salt, for example the aqueous formulation does not comprise any one of histidine malate, histidine maleate, histidine fumarate, histidine tartrate, histidine phosphate, histidine lactate, histidine succinate and/or histidine hydrochloride. In some embodiments, the aqueous formulation does not comprise histidine hydrochloride. In some embodiments, the aqueous formulation does not comprise histidine hydrochloride and histidine.

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Trehalose, sucrose, mannitol, sorbitol, methionine, histidine, glycine, arginine, and combinations thereof are usually used as stabilisers in formulations, for example protein formulations. In some embodiments,

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the aqueous formulation does not comprise sucrose. In some embodiments, the aqueous formulation does not comprise trehalose, sucrose, mannitol, sorbitol, methionine, histidine, glycine and/or arginine.

5 In some embodiments, the aqueous formulation does not comprise phosphate, for example, the aqueous formulation does not comprise sodium phosphate and/or potassium phosphate.

10 In some embodiments, the aqueous formulation substantially lacks histidine. For example, the aqueous formulation substantially lacks histidine buffer. In some embodiments, the aqueous formulation substantially lacks a histidine buffer salt, for example the aqueous formulation substantially lacks any one of histidine malate, histidine maleate, histidine fumarate, histidine tartrate, histidine citrate, histidine phosphate, histidine lactate, histidine succinate and/or histidine hydrochloride. In some embodiments, the aqueous formulation substantially lacks histidine hydrochloride. In some embodiments, the aqueous formulation substantially lacks histidine hydrochloride and histidine.

15 In some embodiments, the aqueous formulation substantially lacks sucrose. In some embodiments, the aqueous formulation substantially lacks trehalose, sucrose, mannitol, sorbitol, methionine, histidine, glycine and/or arginine.

20 In some embodiments, the aqueous formulation substantially lacks phosphate, for example, the aqueous formulation substantially lacks sodium phosphate and/or potassium phosphate.

25 As used herein, within a composition (e.g. an aqueous formulation) which 'substantially lacks' one or more specified compound(s) (e.g. histidine, or sucrose *etc.*), said compound(s) may not be present at greater than trace amounts. In some embodiments, within a composition (e.g. an aqueous formulation) which 'substantially lacks' one or more specified compound(s), said compound(s) may be present at less than 5 mM, e.g. one of ≤ 1 mM, ≤ 0.5 mM, ≤ 0.1 mM, ≤ 0.05 mM or ≤ 0.01 mM. By way of illustration, in an aqueous formulation according to the present disclosure substantially lacking a histidine buffer salt, the histidine buffer salt may have a concentration of less than 5 mM, e.g. one of ≤ 1 mM, ≤ 0.5 mM, ≤ 0.1 mM, ≤ 0.05 mM or ≤ 0.01 mM in the aqueous formulation.

30 In some embodiments, the aqueous formulation does not consist essentially of: (i) 10 mM L-histidine/L-histidine hydrochloride (L-His/L-His HCl); and (ii) 0.02% (w/v) polysorbate 80.

35 In some embodiments, the aqueous formulation does not consist essentially of: (i) 10 mM L-histidine/L-histidine hydrochloride; and (ii) 0.02% (w/v) polysorbate 80; and (iii) Fe^{2+} at a concentration of any one of: 0.1, 1, or 10 ppm.

40 In some embodiments, the aqueous formulation does not consist essentially of: (i) 10 mM sodium phosphate monobasic monohydrate/sodium phosphate dibasic anhydrous; and (ii) 0.02% (w/v) polysorbate 80.

In some embodiments, the aqueous formulation does not consist essentially of: (i) 25 mM L-His/L-His HCl; (ii) 600 mM sucrose; and (iii) 0.05 % (w/v) polysorbate 80.

5 In some embodiments, the aqueous formulation does not consist essentially of: (i) 10.5 mM L-His/L-His HCl, (ii) 250 mM sucrose; (iii) 0.02 % (w/v) polysorbate 80, and (iv) 1.4 mg/mL Rituximab. Rituximab (DrugBank Acc. No. DB00073) is a monoclonal anti-CD20 antibody.

10 In some embodiments, the aqueous formulation does not consist essentially of: (i) 10.5 mM L-His/L-His HCl, (ii) 250 mM sucrose; and (iii) 0.02 % (w/v) polysorbate 80.

15 It will be appreciated that citrate is added to the aqueous formulation to prevent degradation of polysorbate (e.g. PS80) in the aqueous formulation. The final concentration of citrate in the aqueous formulation may therefore depend on the concentration of polysorbate (e.g. PS80). It will be appreciated that the concentration of citrate and the concentration of polysorbate (e.g. PS80) in the aqueous formulation is selected in accordance with molar ratios of citrate to polysorbate (e.g. PS80) described herein.

20 Accordingly, it will be appreciated that citrate is added to the aqueous formulation to prevent degradation of polysorbate 20 in the aqueous formulation. The final concentration of citrate in the aqueous formulation may therefore depend on the concentration of polysorbate 20. It will be appreciated that the concentration of citrate and the concentration of polysorbate 20 in the aqueous formulation is selected in accordance with molar ratios of citrate to polysorbate 20 described herein.

25 In some embodiments, 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 (e.g. concentrations ≥ 5 mM) may, e.g., result in formation of protein particles, if a protein or peptide is added, and/or promote gelation. In some embodiments, the concentration of citrate in the aqueous formulation is from about 50 μ M to about 2.5 mM. In some embodiments, the concentration of citrate in the aqueous formulation is from about 250 μ M to about 1 mM. In some embodiments, the concentration of citrate in the aqueous formulation is from about 0.5 mM to about 1 mM.

30 In some embodiments, the concentration of citrate is less than 2.5 mM. In contrast thereto, the concentration of citrate acting as buffer, e.g. in protein formulations, is usually between 10 mM and 20 mM, while at concentrations below e.g. 2.5 mM, citrate is usually not able to act as a buffer in the aqueous formulation. Accordingly, citrate preferably does not act as a buffer in the aqueous formulation. Preferably, citrate acts as a stabilizer in the aqueous formulation.

35 In some embodiments, the concentration of citrate in the aqueous formulation is about 0.25 mM. In some embodiments, the concentration of citrate in the aqueous formulation is about 0.50 mM. In some embodiments, the concentration of citrate in the aqueous formulation is about 0.75 mM. In some embodiments, the concentration of citrate in the aqueous formulation is about 1 mM.

In some embodiments, the concentration of citrate in the aqueous formulation is from about 250 μ M to about 1 mM. In some embodiments, the concentration of citrate in the aqueous formulation is from about 250 μ M to about 750 μ M. In some embodiments, the concentration of citrate in the aqueous formulation is from about 250 μ M to about 0.5 mM. In some preferred embodiments, the concentration of citrate in the aqueous formulation is about 0.5 mM.

Aqueous formulations according to the present disclosure may further comprise an agent of interest, *e.g.* an agent useful in therapy, prophylaxis and/or diagnosis. Such agents include biomolecules, *e.g.* proteins (e.g. peptides/polypeptides, complexes thereof), glycoproteins, lipoproteins, nucleic acids (e.g. polynucleotides/oligonucleotides), sugars, lipids (fatty acids, glycerides) phospholipids, glycolipids, *etc.*, and complexes thereof. In some embodiments, an aqueous formulation according to the present disclosure comprises a peptide/polypeptide or complex thereof for use in therapy, prophylaxis and/or diagnosis (e.g. as described hereinbelow).

In some embodiments, the concentration of the agent (e.g. peptide/polypeptide) in an aqueous formulation/composition according to the present disclosure is from about 1×10^{-5} mg/mL to about 15 mg/mL, *e.g.* one of: from about 1×10^{-5} mg/mL to about 1×10^{-4} mg/mL, about 1×10^{-4} mg/mL to about 0.01 mg/mL, from about 0.01 mg/mL to about 0.1 mg/mL, from about 0.1 mg/mL to about 1 mg/mL, from about 1 mg/mL to about 5 mg/mL, from about 5 mg/mL to about 10 mg/mL, and from about 10 mg/mL to about 15 mg/mL. In some embodiments, the concentration of the agent is about 1×10^{-5} mg/mL, about 1×10^{-4} mg/mL, about 0.01 mg/mL, about 0.1 mg/mL, about 1 mg/mL, about 5 mg/mL, about 10 mg/mL or about 15 mg/mL. In some embodiments, the concentration of the agent in the aqueous formulation/composition according to the present disclosure is at least 0.1 mg/mL, at least 1 mg/mL, at least 5 mg/mL or at least 10 mg/mL. In some embodiments, the concentration of the agent in the aqueous formulation/composition according to the present disclosure is about 15 mg/mL, about 20 mg/mL, about 25 mg/mL or about 30 mg/mL. In some embodiments, the concentration of the agent (e.g. peptide/polypeptide) in an aqueous formulation/composition according to the present disclosure is from about 10 mg/mL to about 100 mg/mL, *e.g.* one of: from about 10 mg/mL to about 20 mg/mL, from about 20 mg/mL to about 30 mg/mL, from about 30 mg/mL to about 40 mg/mL, from about 40 mg/mL to about 50 mg/mL, from about 50 mg/mL to about 60 mg/mL, from about 60 mg/mL to about 70 mg/mL, from about 70 mg/mL to about 80 mg/mL, from about 80 mg/mL to about 90 mg/mL, and from about 90 mg/mL to about 100 mg/mL. Preferably, the concentration of the agent (e.g. peptide/polypeptide) in an aqueous formulation/composition according to the present disclosure is from about 10 mg/mL to about 75 mg/mL. More preferably, the concentration of the agent (e.g. peptide/polypeptide) in an aqueous formulation/composition according to the present disclosure is from about 10 mg/mL to about 50 mg/mL. Even more preferably, the concentration of the agent (e.g. peptide/polypeptide) in an aqueous formulation/composition according to the present disclosure is from about 10 mg/mL to about 30 mg/mL. Still more preferably, the concentration of the agent (e.g. peptide/polypeptide) in an aqueous formulation/composition according to the present disclosure is from about 10 mg/mL to about 25 mg/mL. In some embodiments, the concentration of the agent in the aqueous formulation/composition according

to the present disclosure is about 35 mg/mL, about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, or about 70 mg/mL. In some embodiments, the concentration of the agent is 50 mg/mL. In some embodiments, the concentration of the agent in the aqueous formulation/composition according to the present disclosure is about 75 mg/mL, about 80
5 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, or about 100 mg/mL. In some embodiments, the concentration of the agent is 100 mg/mL.

In some embodiments, the aqueous formulation consists essentially of:

- about 0.2 mg·mL⁻¹ polysorbate (e.g. PS80 or PS20 etc.),
- 10 - about 0.9 % (w/v) sodium chloride,
- water,
- 0.25 mM to 1 mM, preferably 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, optionally at a
15 concentration as defined hereinabove.

Preferably, the aqueous formulation consists essentially of:

- about 0.2 mg·mL⁻¹ polysorbate 20 (PS20),
- about 0.9 % (w/v) sodium chloride,
- 20 - water, and
- 0.25 mM to 1 mM, preferably about 0.5 mM, citrate.

Prevention of degradation of polysorbate

The present disclosure provides aqueous formulations in which degradation of polysorbate (e.g. polysorbate 80 or polysorbate 20) is prevented. That is, the polysorbate is stable in the aqueous
25 formulations such that the level of intact polysorbate is not significantly reduced in the aqueous formulations (e.g. during storage and/or exposure to light).

In some aspects, the present disclosure provides aqueous formulations in which degradation of PS20 is prevented. That is, PS20 is stable in the aqueous formulations such that the level of intact PS20 is not
30 significantly reduced in the aqueous formulations (e.g. during storage).

In some embodiments, degradation of polysorbate comprises oxidation of polysorbate (e.g. polysorbate 80). In some embodiments, degradation of polysorbate 20 comprises oxidation of polysorbate 20.
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Oxidative pathways target the hydrocarbon chain double bonds of unsaturated fatty acids and/or the polyoxyethylene (POE) chains of polysorbate 20, and are summarised by Donbrow *et al.* (26). Polysorbate 80 oxidation involves two oxidation pathways. The head group of the non-ionic surfactant is oxidised by free radical species, resulting in fragmentation of the head group. Free radical species may
40 also oxidise any unsaturated fatty acids attached to the head group, leading to various degradation and fragmentation species Gopalrathnam *et al.* (2018) (47). Oxidative degradation may occur as a result of

reactive oxygen species (ROS) being present in the aqueous formulation. ROS may be generated in the presence of hydrogen peroxide (H₂O₂).

5 In some embodiments, degradation of polysorbate (e.g. PS80, PS20 *etc.*) comprises degradation as a result of exposure to light (e.g. photooxidation).

10 In some embodiments, degradation of polysorbate (e.g. PS80) comprises degradation of in the presence of a peptide/polypeptide or complex thereof described herein, for example in a composition described herein.

10 In some embodiments, degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a peptide/polypeptide or complex thereof described herein, for example in a composition described herein.

15 In some embodiments, degradation of polysorbate (e.g. polysorbate 80) comprises degradation of polysorbate (e.g. polysorbate 80) in the presence of a metal ion. In some embodiments, degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a metal ion. Thus, in some embodiments, degradation of PS20 comprises degradation mediated by metal ion-dependent hydroxyl radical generation. Metal ions such as copper and iron ions are known to be involved in Fenton-type ROS
20 generation. In certain embodiments, the metal ion is iron ion, e.g. Fe²⁺.

The metal ion may be derived from any substance/molecule comprising a metal ion. In some embodiments, the source of a metal ion may be a vessel/container or support for the aqueous formulation. For example, the source of the metal ion may be a metal ion containing vessel/container, e.g.
25 a stainless steel vessel/container in which the aqueous formulation is prepared/contained/stored/provided. In some embodiments, the source of a metal ion may be a carrier, excipient or adjuvant employed with, or comprised in, the aqueous formation.

30 In some embodiments, preventing degradation of polysorbate (e.g. PS80) comprises preventing degradation of (e.g. PS80) in an aqueous formulation or composition in a vessel/container comprising or made of stainless steel. In some embodiments, preventing degradation of PS20 comprises preventing degradation of PS20 in an aqueous formulation or composition in a vessel/container comprising or made of stainless steel. In some embodiments, the aqueous formulation or composition is in a container (e.g. a syringe) which comprises a stainless steel needle.

35 In some embodiments, the aqueous formulation or composition is in a glass vessel/container, for example a glass vial such as a type I borosilicate glass vial.

40 In some embodiments, the aqueous formulation is provided in a vessel/container comprising or made of a plastic. Plastics generally comprise organic polymers, and include acrylics, polyesters, silicones, polyurethanes and halogenated plastics. In some embodiments, a plastic according to the present

disclosure is or comprises a vinyl polymer or a vinyl copolymer, polyethylene (PE), polypropylene (PP), polystyrene, polyvinyl chloride (PVC), a polyvinyl ester, polyvinyl acetate (PVAc), polyacrylonitrile, polyolefin (PP + PE) and ethyl-vinyl acetate (EVA; copolymer of ethylene and vinyl acetate). In some embodiments, the aqueous formulation is provided in a vessel/container comprising or made of EVA; for example, the container may be provided in an infusion bag, such as a Flexboy® bag.

Preventing degradation of polysorbate (e.g. PS80) may comprise maintaining the level of (intact) polysorbate in the aqueous formulation over a specified period of time. An exemplary LCMS-based method for quantifying the level of PS80 is described in Gopalrathnam *et al.* (2018) (47), which is incorporated by reference in its entirety. An exemplary method for quantifying the level of a polysorbate is a fluorescence micelle assay (FMA). Such an assay is described by Lippold *et al.* (2017) (41), which is hereby incorporated by reference in its entirety.

Preventing degradation of PS20 may comprise maintaining the level of (intact) PS20 in the aqueous formulation over a specified period of time. An exemplary method for quantifying the level of PS20 is a fluorescence micelle assay (FMA). Such an assay is described by Lippold *et al.* (2017) (41), which is hereby incorporated by reference in its entirety.

In some embodiments, the period of time is from about 2 weeks to about 36 months, e.g. one of: from about 2 weeks to about 1 month, from about 1 month to about 2 months, from about 2 months to about 3 months, from about 3 months to about 4 months, from about 4 months to about 5 months, from about 5 months to about 6 months, from about 6 months to about 7 months, from about 7 months to about 8 months, from about 8 months to about 9 months, from about 9 months to about 10 months, from about 11 months to about 12 months, from about 12 months to about 15 months, from about 15 months to about 18 months, from about 18 months to about 21 months, from about 21 months to about 24 months, from about 24 months to about 27 months, from about 27 months to about 30 months, from about 30 months to about 33 months, or from about 33 months to about 36 months. In some embodiments, the period of time is at least about 2 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 15 months, at least about 18 months, at least about 21 months, at least about 24 months, at least about 27 months, at least about 30 months, at least about 33 months, or at least about 36 months.

In some embodiments, preventing degradation of PS20 comprises maintaining the level of PS20 in the aqueous formulation for a given period of time (e.g. a period of time described herein), at a given temperature. In some embodiments, preventing degradation of polysorbate (e.g. PS80) comprises essentially maintaining the level of polysorbate (e.g. PS80) in the aqueous formulation for a given period of time (e.g. a period of time described herein), at a given temperature. In some embodiments, the given temperature is from about 2°C to about 60°C, e.g. one of: from about 2°C to about 5°C, from about 5°C to about 10°C, from about 10°C to about 15°C, from about 15°C to about 20°C, from about 20°C to about

25°C, from about 25°C to about 30°C, from about 30°C to about 35°C, from about 35°C to about 40°C, from about 40°C to about 45°C, from about 45°C to about 50°C, from about 50°C to about 55°C, or from about 55°C to about 60°C. In some embodiments, the temperature is about 2°C, about 5°C, about 10°C, about 15°C, about 20°C, about 25°C, about 30°C, about 35°C, about 40°C, about 45°C, about 50°C,
5 about 55°C, or about 60°C.

In some embodiments, maintaining the level of PS20 comprises maintaining at least 95%, at least 90% or at least 85% of the initial amount of PS20 in the aqueous formulation or composition. In some
10 embodiments, maintaining the level of polysorbate (e.g. PS80) comprises maintaining at least 95%, at least 90% or at least 85% of the initial amount of polysorbate (e.g. PS80) in the aqueous formulation or composition.

In some embodiments, the aqueous formulation or composition comprises hydrogen peroxide (such that degradation of polysorbate, in particular PS20, is prevented in the presence of hydrogen peroxide). In
15 some embodiments, the concentration of hydrogen peroxide is from about 1 ppm to about 1000 ppm, for example one of from: 1 ppm to 100 ppm, 100 ppm to 250 ppm, 250 ppm to 500 ppm, 500 ppm to 750 ppm, and 750 ppm to 1000 ppm. In some embodiments, the concentration of hydrogen peroxide is 1, 100, 250, 500, 750 or 1000 ppm. However, H₂O₂ is usually not required. In the appended examples, H₂O₂ was added to enhance the stress during the stability studies. Preferably, the aqueous formulation or
20 composition does not comprise hydrogen peroxide.

In some embodiments, the aqueous formulation or composition comprises at least 95%, at least 90%, or at least 85% of the initial amount of polysorbate, in particular PS20, after incubation for a period of from about 0.5 months to about 12 months at no more than 40°C, such as about 40°C. In some embodiments,
25 the aqueous formulation or composition comprises at least 95%, at least 90%, or at least 85% of the initial amount of polysorbate, in particular PS20, after incubation for a period of from about 0.5 months to about 12 months at about 25°C. In some embodiments, the aqueous formulation or composition comprises at least 95%, at least 90%, or at least 85% of the initial amount of polysorbate, in particular PS20 after incubation at no more than 40°C, e.g. at about 40°C or at about 25°C, for one of: about 2 weeks to about
30 1 month, about 1 month to about 2 months, about 2 months to about 3 months, about 3 months to about 4 months, about 4 months to about 5 months, about 5 months to about 6 months, about 6 months to about 7 months, about 7 months to about 8 months, about 8 months to about 9 months, about 9 months to about 10 months, and about 11 months to about 12 months. In some embodiments, the period is at least about 2 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4
35 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, or at least about 12 months.

In some embodiments, the aqueous formulation or composition comprises at least 95% of the initial
40 amount of polysorbate, in particular PS20, after a period of about 1 month, about 2 months, about 3 months, about 4 months, about 6 months or about 12 months, for example under the conditions specified

hereinabove (such as temperature, and/or hydrogen peroxide presence). In some embodiments, the aqueous formulation or composition comprises at least 90% of the initial amount of polysorbate, in particular PS20, after a period of about 1 month, about 2 months, about 3 months, about 4 months, about 6 months or about 12 months, for example under the conditions specified hereinabove (such as temperature, and/or hydrogen peroxide presence). In some embodiments, the aqueous formulation or composition comprises at least 85% of the initial amount of polysorbate, in particular PS20, after a period of 1 month, about 2 months, about 3 months, about 4 months, about 6 months or about 12 months, for example under the conditions specified hereinabove (such as temperature, and/or hydrogen peroxide presence).

In some embodiments, the aqueous formulation or composition is provided in a container as described herein. In some embodiments, the aqueous formulation or composition is stored at relative humidity of about 75%.

In some embodiments, preventing degradation of PS20 comprises reducing the levels of oxidation products of PS20 in an aqueous formulation of the present disclosure as compared to the levels of an aqueous formulation not comprising citrate. In some embodiments, the oxidation products comprise one or more of: the mono-ester of laurate with 5 POE subunits (POE5 Mono), the di-ester of formate and laurate with 5 POE subunits (POE5 Di), the mono-ester of laurate with 6 POE subunits (POE6 Mono) and di-ester of formate and laurate with 6 POE subunits (POE6 Di). The structures of these oxidation markers are shown in Table 3. Suitable methods for quantifying oxidation products include Ultra High Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS).

Oxidation products of PS80 are reported in Hvattum *et al.* (2012) (49) and Borisov *et al.* (2015) (50), the disclosures of which are incorporated herein by reference in their entirety. In some embodiments, preventing degradation of PS80 comprises reducing the levels of oxidation products of PS80 in an aqueous formulation of the present disclosure as compared to the levels of an aqueous formulation not comprising citrate. In some embodiments, the oxidation products comprise 9-oxo-C9:0-ester and/or Hydroxy-C18:1-ester. Suitable methods for quantifying oxidation products include Ultra High Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS).

Thus, the aqueous formulations, or compositions obtained by the methods of the present disclosure may exhibit one or both of the following properties, compared to an aqueous formulation or composition not comprising citrate, and maintained under the same conditions (*e.g.* at the same temperature, in the same relative humidity, in the presence of the same quantity concentration of an oxidising agent, *etc.*):

comprise a higher level of PS20 over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;

comprise lower levels of oxidation markers of PS20, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months.

Moreover, the aqueous formulations, or compositions obtained by the methods of the present disclosure may exhibit one or more of the following properties, compared to an aqueous formulation or composition not comprising citrate, and maintained under the same conditions (*e.g.* at the same temperature, in the same relative humidity, in the presence of the same quantity concentration of an oxidising agent, under exposure to light *etc.*):

- 5 comprise a higher level of polysorbate over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- comprise lower levels of oxidation markers of polysorbate, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- 10 comprise a higher level of PS80 over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- comprise lower levels of oxidation markers of PS80, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- have the same level of turbidity, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- 15 comprise significantly the same concentration of microparticles, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- comprise a lower concentration of microparticles, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months;
- 20 maintain the level of photostability of polysorbate and/or polypeptide in the aqueous formulation, optionally over a specified period of time, such as 1, 2, 3, 4, 6, 12, 18, 24 or 36 months.

Compositions

- As described herein, the aqueous formulation may be used as a dilution medium for a composition. A dilution medium, or diluent, is a solution used to prepare solutions or formulations of a composition or a peptide/polypeptide or complex thereof, such as an antibody or an antigen-binding fragment thereof. For example, a dilution medium may be used to prepare a composition for administration.

- The compositions of the present disclosure may comprise a peptide/polypeptide or complex thereof described herein, in particular an antibody (*i.e.* an immunoglobulin) or an antigen-binding fragment thereof. In some embodiments, the compositions are provided in lyophilised form. In this case, the aqueous formulation may be used to resuspend the lyophilised composition. Alternatively, the lyophilised composition may be resuspended first in a distinct medium and, thereafter, diluted with the aqueous formulation as described herein; *e.g.* to provide the peptide/polypeptide or complex thereof (*e.g.* the antibody or an antigen-binding fragment thereof), at the desired concentration for administration. In some embodiments, the composition comprises an agent of interest (*e.g.* peptide/polypeptide) thereof at a concentration as specified hereinabove.

- In some aspects, the disclosure provides a composition comprising polysorbate (*e.g.* PS80, PS20 *etc.*), citrate and sodium chloride, wherein the molar ratio of citrate to polysorbate in the composition is from about 1.5 to about 7, wherein the composition is provided in lyophilised form. In some embodiments, the

molar ratio is from about 1.6 to about 7. In some embodiments, the molar ratio is from about 3 to about 6. In preferred embodiments, the molar ratio of citrate to polysorbate in the composition 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 some embodiments, the composition does not comprise histidine. In some embodiments, the composition further comprises a metal ion. In some embodiments, the composition further comprises a pharmaceutically-acceptable carrier, excipient or adjuvant. In some embodiments, the composition is provided in a glass container, a stainless steel container or a container comprising a plastic. The composition may further comprise a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis. In some embodiments, 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.

The disclosure further 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 the aqueous formulation. The peptide/polypeptide or complex thereof described herein may be formulated as pharmaceutical compositions or medicaments for clinical use and may comprise a pharmaceutically-acceptable carrier, excipient or adjuvant.

In some embodiments, the aqueous formulation or composition comprises a pharmaceutically-acceptable carrier, excipient or adjuvant.

The compositions/aqueous formulations of the present disclosure may comprise one or more pharmaceutically-acceptable carriers (e.g. liposomes, micelles, microspheres, nanoparticles), excipients (e.g. starch, cellulose, a cellulose derivative, a polyol, dextrose, maltodextrin, magnesium stearate), adjuvants, fillers, buffers, preservatives (e.g. vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium, cysteine, methionine, methyl paraben, propyl paraben), anti-oxidants (e.g. vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium), lubricants (e.g. magnesium stearate, talc, silica, stearic acid, vegetable stearin), binders (e.g. sucrose, lactose, starch, cellulose, gelatin, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), xylitol, sorbitol, mannitol), stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents or colouring agents (e.g. titanium oxide). In some embodiments, the aqueous formulation/composition does not comprise sucrose. In some embodiments, the aqueous formulation/composition does not comprise trehalose, sucrose, mannitol, sorbitol, methionine, histidine, glycine and/or arginine.

The term 'pharmaceutically-acceptable' as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, *etc.*, which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g. a human subject) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, adjuvant, filler, buffer, preservative, anti-oxidant, lubricant,

binder, stabiliser, solubiliser, surfactant, masking agent, colouring agent, flavouring agent or sweetening agent of a composition according to the present disclosure must also be 'acceptable' in the sense of being compatible with the other ingredients of the formulation. Suitable carriers, excipients, adjuvants, fillers, buffers, preservatives, anti-oxidants, lubricants, binders, stabilisers, solubilisers, surfactants, masking agents, colouring agents, flavouring agents or sweetening agents can be found in standard pharmaceutical texts, for example, Remington's 'The Science and Practice of Pharmacy' (Ed. A. Adejare), 23rd Edition (2020), Academic Press.

Compositions (e.g. pharmaceutical compositions) of the present disclosure may be formulated or prepared according to the methods of the present disclosure for intravenous, parenteral, systemic, intracavitary, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, subconjunctival, intravitreal, intratumoral, subcutaneous, intradermal, oral or transdermal administration. In some embodiments, a composition may be formulated/prepared for administration by injection or infusion, or administration by ingestion. Accordingly, aqueous formulations of the present disclosure may be suitable for intravenous, parenteral, systemic, intracavitary, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, subconjunctival, intravitreal, intratumoral, subcutaneous, intradermal, oral or transdermal administration. In some embodiments, an aqueous formulation of the disclosure is suitable for administration by injection or infusion.

In some embodiments, the composition (e.g. pharmaceutical composition) is formulated/prepared for injection or infusion, e.g. into a blood vessel, tissue/organ of interest, or a tumor.

In some embodiments, the composition is prepared by dilution in the aqueous formulation described herein. An appropriate dilution may be used according to the subsequent use of the composition. For example, in some embodiments, the composition is diluted 10-fold, 100-fold, 10³-fold, 10⁴-fold, 10⁵-fold or 10⁶-fold. Preparation of a composition for administration by infusion, for example, may require a dilution factor of e.g. 100-fold.

The present disclosure also provides methods for the production of pharmaceutically useful compositions, such methods of production may comprise one or more steps selected from:

- producing a peptide/polypeptide or complex thereof for use in therapy, prophylaxis and/or diagnosis described herein; and/or
- mixing the peptide/polypeptide or complex thereof with an aqueous formulation described herein.

Agents

The aqueous formulation of the present disclosure is useful as a diluent for an agent of interest, e.g. an agent useful in therapy, prophylaxis and/or diagnosis described hereinabove.

In some embodiments, the agent is a peptide/polypeptide or complex thereof suitable for use in therapy, prophylaxis and/or diagnosis. The peptide/polypeptide or complex thereof may be comprised in a

composition as described herein, and/or contacted with the aqueous formulation of the present disclosure using the methods described herein.

5 A peptide/polypeptide or complex thereof suitable for use in therapy, prophylaxis and/or diagnosis is any peptide/polypeptide (e.g. protein) or complex thereof which has a therapeutic, prophylactic or diagnostic property. Such peptides/polypeptides and peptide/polypeptide complexes are known to the skilled person, for example Dimitrov *et al.* Methods Mol Biol. 2012, 899: 1-26 (hereby incorporated by reference in its entirety) provides a review of proteins suitable for use in therapy/prophylaxis.

10 Peptide/polypeptide complexes are characterised by protein:protein interaction between their constituent polypeptide(s)/peptide(s). In some embodiments, the association comprises non-covalent (electrostatic interaction (e.g. ionic bonding, hydrogen bonding) and/or Van der Waals forces) and/or covalent interaction (e.g. disulfide bonding). By way of illustration peptide/polypeptide complexes contemplated in accordance with the present disclosure include IgG antibodies, which comprise four polypeptide chains
15 that associate via protein:protein interaction to form a polypeptide complex.

In some embodiments, the peptide/polypeptide or complex thereof 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
20 enzyme, a growth factor, a hormone, an interferon, an interleukin, and a thrombolytic.

Antigen-binding peptides/polypeptides and antigen-binding peptide/polypeptide complexes refer to peptides/polypeptides and peptide/polypeptide complexes that bind to a given target antigen. Such peptides/polypeptides and peptide/polypeptide complexes include antibodies (*i.e.* immunoglobulins, including monoclonal antibodies, polyclonal antibodies, monospecific and multispecific (e.g., bispecific, trispecific, *etc.*) antibodies), antibody fragments (including antigen-binding fragments, such as Fv, Fab, F(ab')₂ and F(ab') fragments) and antibody-derived molecules (including scFv, scFab, diabodies, triabodies, scFv-Fc, minibodies, single domain antibodies (e.g. VhH), *etc.*).
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30 In some embodiments, the antigen-binding polypeptide is a monoclonal antibody.

In some embodiments, an antigen-binding peptide/polypeptide comprises or consists of a peptide aptamer, thioredoxin, monobody, anticalin, Kunitz domain, avimer, knottin, fynomer, atrimer, DARPin, affibody, nanobody (*i.e.* a single-domain antibody (sdAb)), affilin, armadillo repeat protein (ArmRP),
35 Obody or fibronectin – reviewed e.g. in Reverdatto *et al.*, Curr Top Med Chem. 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also e.g. Boersma *et al.*, J Biol Chem (2011) 286:41273-85 and Emanuel *et al.*, Mabs (2011) 3:38-48).

In some embodiments, an antigen-binding polypeptide comprises, or consists of, the antigen-binding
40 region of an antibody (e.g. an antigen-binding fragment of an antibody). Antigen-binding polypeptides of the present disclosure preferably comprise the antibody heavy chain variable region (VH) and the

antibody light chain variable region (VL) of an antibody that binds to the target antigen. The antigen-binding domain formed by a VH and a VL may also be referred to herein as an Fv region.

5 In some embodiments, the antigen-binding polypeptide is or comprises the Fv (e.g. provided as an scFv) of an antibody. In some embodiments, the antigen-binding polypeptide is or comprises the Fab region of an antibody. In some embodiments, the antigen-binding polypeptide is or comprises the whole antibody (i.e. comprising variable and constant regions).

10 An antigen-binding polypeptide may be, or may comprise, an antigen-binding polypeptide complex. An antigen-binding polypeptide may comprise more than one polypeptide which together form an antigen-binding moiety. The polypeptides may associate covalently or non-covalently. In some embodiments, the polypeptides form part of a larger polypeptide comprising the polypeptides (e.g. in the case of scFv comprising VH and VL, or in the case of scFab comprising VH-CH1 and VL-CL).

15 An antigen-binding polypeptide may refer to a non-covalent or covalent complex of more than one polypeptide (e.g. 2, 3, 4, 6, or 8 polypeptides), e.g. an IgG-like antigen-binding polypeptide comprising two heavy chain polypeptides and two light chain polypeptides.

20 The antigen-binding polypeptides of the present disclosure may be designed and prepared using the sequences of monoclonal antibodies (mAbs) capable of binding to a given target antigen. Antigen-binding regions of antibodies, such as single chain variable fragment (scFv), Fab and F(ab')₂ fragments may also be used/provided. An 'antigen-binding region' is any fragment of an antibody that binds to the target for which the given antibody is specific.

25 Antibodies generally comprise six complementarity-determining regions CDRs; three in the heavy chain variable (VH) region: HC-CDR1, HC-CDR2 and HC-CDR3, and three in the light chain variable (VL) region: LC-CDR1, LC-CDR2, and LC-CDR3. The six CDRs together define the paratope of the antibody, which is the part of the antibody that binds to the target antigen.

30 The VH region and VL region comprise framework regions (FRs) either side of each CDR, which provide a scaffold for the CDRs. From N-terminus to C-terminus, VH regions comprise the following structure: N term-[HC-FR1]-[HC-CDR1]-[HC-FR2]-[HC-CDR2]-[HC-FR3]-[HC-CDR3]-[HC-FR4]-C term; and VL regions comprise the following structure: N term-[LC-FR1]-[LC-CDR1]-[LC-FR2]-[LC-CDR2]-[LC-FR3]-[LC-CDR3]-[LC-FR4]-C term.

35 The VL and light chain constant (CL) region, and the VH region and heavy chain constant 1 (CH1) region of an antigen-binding region of an antibody together constitute the Fab region. In some embodiments, the antigen-binding polypeptide comprises a Fab region comprising a VH, a CH1, a VL and a CL (e.g. C_κ or C_λ). In some embodiments, the Fab region comprises a polypeptide comprising a VH and a CH1 (e.g. a VH-CH1 fusion polypeptide), and a polypeptide comprising a VL and a CL (e.g. a VL-CL fusion polypeptide). In some embodiments, the Fab region comprises a polypeptide comprising a VH and a CL

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(e.g. a VH-CL fusion polypeptide) and a polypeptide comprising a VL and a CH (e.g. a VL-CH1 fusion polypeptide); that is, in some embodiments, the Fab region is a CrossFab region. In some embodiments, the VH, CH1, VL and CL regions of the Fab or CrossFab are provided as single polypeptide joined by linker regions, *i.e.* as a single chain Fab (scFab) or a single chain CrossFab (scCrossFab).

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In some embodiments, an antigen-binding polypeptide described herein comprises, or consists of, a whole antibody. As used herein, 'whole antibody' refers to an antibody having a structure which is substantially similar to the structure of an immunoglobulin (Ig). Different kinds of immunoglobulins and their structures are described e.g. in Schroeder and Cavacini J Allergy Clin Immunol. (2010) 125(202): S41-S52, which is hereby incorporated by reference in its entirety.

10

Immunoglobulins of type G (*i.e.* IgG) are ~150 kDa glycoproteins comprising two heavy chains and two light chains. From N- to C-terminus, the heavy chains comprise a VH followed by a heavy chain constant region comprising three constant domains (CH1, CH2, and CH3), and similarly the light chains comprise a VL followed by a CL. Depending on the heavy chain, immunoglobulins may be classed as IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE, or IgM. The light chain may be kappa (κ) or lambda (λ).

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In some embodiments, the antigen-binding polypeptide comprises, or consists of, an IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE, or IgM.

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In some embodiments, the antigen-binding polypeptides of the present disclosure comprise an Fc region.

As used herein, an 'Fc region' refers to a polypeptide complex formed by interaction between two polypeptides, each polypeptide comprising the CH2-CH3 region of an immunoglobulin (Ig) heavy chain constant sequence.

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Herein, a 'CH2 domain' refers to an amino acid sequence corresponding to the CH2 domain of an immunoglobulin (Ig). The CH2 domain is the region of an Ig formed by positions 231 to 340 of the immunoglobulin constant domain, according to the EU numbering system described in Edelman *et al.*, Proc Natl Acad Sci USA (1969) 63(1): 78-85. A 'CH3 domain' refers to an amino acid sequence corresponding to the CH3 domain of an immunoglobulin (Ig). The CH3 domain is the region of an Ig formed by positions 341 to 447 of the immunoglobulin constant domain, according to the EU numbering system described in Edelman *et al.*, Proc Natl Acad Sci USA (1969) 63(1): 78-85. A 'CH2-CH3 region' refers to an amino acid sequence corresponding to the CH2 and CH3 domains of an immunoglobulin (Ig). The CH2-CH3 region is the region of an Ig formed by positions 231 to 447 of the immunoglobulin constant domain, according to the EU numbering system described in Edelman *et al.*, Proc Natl Acad Sci USA (1969) 63(1): 78-85.

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In some embodiments, a CH2 domain, CH3 domain and/or a CH2-CH3 region according to the present disclosure corresponds to the CH2 domain/CH3 domain/CH2-CH3 region of an IgG (e.g. IgG1, IgG2,

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IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM. In some embodiments, the CH2 domain, CH3 domain and/or a CH2-CH3 region corresponds to the CH2 domain/CH3 domain/CH2-CH3 region of a human IgG (e.g. hIgG1, hIgG2, hIgG3, hIgG4), hIgA (e.g. hIgA1, hIgA2), hIgD, hIgE or hIgM. In some embodiments, the CH2 domain, CH3 domain and/or a CH2-CH3 region corresponds to the CH2 domain/CH3 domain/CH2-CH3 region of a human IgG1 allotype (e.g. G1m1, G1m2, G1m3 or G1m17).

Fc regions provide for interaction with Fc receptors and other molecules of the immune system to bring about functional effects. Fc-mediated effector functions are reviewed e.g. in Jefferis *et al.*, Immunol Rev 1998 163:59-76 (hereby incorporated by reference in its entirety), and are brought about through Fc-mediated recruitment and activation of immune cells (e.g. macrophages, dendritic cells, neutrophils, basophils, eosinophils, platelets, mast cells, NK cells and T cells) through interaction between the Fc region and Fc receptors expressed by the immune cells, recruitment of complement pathway components through binding of the Fc region to complement protein C1q, and consequent activation of the complement cascade. Fc-mediated functions include Fc receptor binding, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), formation of the membrane attack complex (MAC), cell degranulation, cytokine and/or chemokine production, and antigen processing and presentation.

In some embodiments of the present disclosure, the peptide/polypeptide suitable for use in therapy, prophylaxis and/or diagnosis is an Fc fusion protein. Fc fusion proteins are composed of an immunoglobulin Fc region that is directly linked to another peptide. Different kinds of Fc fusion proteins and their structures are described in e.g. Czajkowsky *et al.* EMBO Mol Med (2012) 4, 1015-1028, which is hereby incorporated by reference in its entirety.

25 Methods and Uses

The present disclosure also provides a method for preventing degradation of polysorbate 20 in an aqueous formulation, the method comprising adding citrate to a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7. Suitable molar ratios of citrate to polysorbate 20, concentrations of PS20, and concentrations of citrate are disclosed hereinabove.

30 The step 'adding citrate' may comprise adding citric acid or a salt thereof. For example, citric acid monohydrate, sodium citrate dihydrate or trisodium citrate may be added, for example to prepare the aqueous formulation or composition according to the disclosure.

35 Also provided is the use of citrate at a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7 to prevent degradation of polysorbate 20 in an aqueous formulation. Suitable molar ratios of citrate to polysorbate 20, concentrations of PS20, and concentrations of citrate are disclosed hereinabove.

In some embodiments, the use or method further comprises:
40 (a) preparing an aqueous formulation according to the disclosure; and

(b) contacting an agent as described herein (e.g. a peptide/polypeptide as described herein) with the aqueous formulation.

5 In some embodiments, step (b) comprises diluting the agent in the aqueous formulation. In some embodiments, step (b) comprises adjusting the concentration of the agent in the aqueous formulation.

The present disclosure further provides a method for preparing an aqueous formulation, wherein the method comprises contacting an aqueous solution comprising polysorbate 20 with citrate to a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7. Suitable final molar ratios of citrate to polysorbate 20, final concentrations of PS20, and final concentrations of citrate are disclosed hereinabove.

10 The present disclosure further provides a method for preparing an aqueous formulation, wherein the method comprises contacting an aqueous solution comprising polysorbate (e.g. PS80) with citrate to a final molar ratio of citrate to polysorbate of about 1.5 to about 7, preferably about 1.6 to about 7. Suitable final molar ratios of citrate to polysorbate, for example citrate to either PS80 or polysorbate 20, final concentrations of PS, for example final concentrations of PS80 or PS20, and final concentrations of citrate are disclosed hereinabove.

20 The uses or methods according to the present disclosure may further comprise incubating, storing or maintaining the aqueous formulation. The aqueous formation may be incubated/stored/maintained for a specified period time, and/or under certain conditions (e.g. at a particular temperature), as described hereinabove.

25 Also provided is the use of the aqueous formulation of the present disclosure as a dilution medium for an agent or composition, e.g. an agent/composition as described herein. That is, in some embodiments the aqueous formulation is provided as a diluent. The use may comprise diluting the agent/composition for administration.

30 The present disclosure further provides a method for preparing a composition, for example, preparing a composition for administration, the method comprising contacting an agent as described herein with an aqueous formulation of the present disclosure.

Kits

35 The present disclosure also provides kits of parts. A kit according to the present disclosure may comprise components for performing a method described herein, in whole or in part.

The kit may have at least one container having a predetermined quantity of an aqueous formulation or composition described herein. Suitable containers are described herein.

In some aspects of the present disclosure a kit of parts is provided. In some embodiments, the kit may comprise an aqueous formulation and/or composition described herein, and which may be provided in a predetermined quantity.

- 5 Also provided is a kit comprising a pharmaceutical composition and a dilution medium for diluting the pharmaceutical composition, wherein the dilution medium is the aqueous formulation of the present disclosure.

- The kit may provide a pharmaceutical composition described herein together with instructions for administration to a patient in order to treat a specified disease/condition (e.g. a disease/condition described herein, e.g. a cancer).
- 10

- The kit may further comprise reagents, buffers and/or standards required for execution of a method according to the present disclosure, e.g. a method for preparing a pharmaceutical composition for administration. Kits according to the present disclosure may include instructions for use, e.g. in the form of an instruction booklet or leaflet. The instructions may include a protocol for performing any one or more of the methods described herein.
- 15

Numbered statements

- 20 The following numbered paragraphs (paras) describe particular aspects and embodiments of the present invention:

1. An aqueous formulation comprising polysorbate 20, citrate and sodium chloride.
- 25 2. The aqueous formulation of para 1, wherein the concentration of citrate in the aqueous formulation is from about 50 μ M to about 2.5 mM.
3. The aqueous formulation of para 1 or para 2, wherein the concentration of citrate in the aqueous formulation is from about 250 μ M to about 1 mM.
- 30 4. The aqueous formulation of any one of paras 1 to 3, wherein the concentration of polysorbate 20 in the aqueous formulation is about 0.02% (w/v).
5. The aqueous formulation of any one of paras 1 to 4, wherein the concentration of sodium chloride in the aqueous formulation is about 0.9 % (w/v).
- 35 6. The aqueous formulation of any one of paras 1 to 5, wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 1.5 to about 7.
- 40 7. The aqueous formulation of any one of paras 1 to 6, wherein the molar ratio of citrate to polysorbate 20 is from about 3 to about 6.

8. The aqueous formulation of any one of paras 1 to 7, wherein the 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.
- 5
9. The aqueous formulation of any one of paras 1 to 8, wherein the aqueous formulation does not comprise histidine.
10. The aqueous formulation of any one of paras 1 to 9, wherein the aqueous formulation further comprises a metal ion.
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11. The aqueous formulation according to any one of paras 1 to 10, wherein the concentration of sodium chloride in the aqueous formulation is about 0.9 % (w/v), and wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 3 to about 6.
- 15
12. The aqueous formulation according to any one of paras 1 to 11, wherein the aqueous formulation consists essentially of polysorbate 20, citrate, water and sodium chloride.
13. The aqueous formulation according to any one of paras 1 to 12, wherein the aqueous formulation consists essentially of:
- 20
- about 0.02% (w/v) polysorbate 20 (PS20),
 - about 0.9 % (w/v) sodium chloride,
 - water, and
 - 0.25 mM to 1 mM citrate.
- 25
14. The aqueous formulation according to para 13, wherein the aqueous formulation consists essentially of:
- about 0.02% (w/v) polysorbate 20 (PS20),
 - about 0.9 % (w/v) sodium chloride,
 - water, and
 - about 0.5 mM citrate.
- 30
15. The aqueous formulation of any one of paras 1 to 14, 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.
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16. The aqueous formulation of any one of paras 1 to 15, further comprising a pharmaceutically-acceptable carrier, excipient or adjuvant.
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17. The aqueous formulation of any one of paras 1 to 16, wherein the aqueous formulation is provided in a glass container, a stainless steel container or a container comprising a plastic.
18. The aqueous formulation of any one of paras 1 to 17, further comprising a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
19. The aqueous formulation of para 18, 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.
20. A method for preventing degradation of polysorbate 20 in an aqueous formulation, the method comprising adding citrate to a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7.
21. The method of para 20, wherein the aqueous formulation comprises sodium chloride.
22. The method of para 20 or 21, wherein the aqueous formulation is as defined in any one of paras 1 to 19.
23. The method of any one of paras 20 to 22, wherein degradation of polysorbate 20 comprises oxidation of polysorbate 20.
24. The method of any one of paras 20 to 23, wherein degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a metal ion.
25. The method of any one of paras 20 to 24, wherein degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
26. The method of para 25, 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.
27. The method of any one of paras 20 to 26, wherein the aqueous formulation is provided in a glass container, a stainless steel container or a container comprising a plastic.
28. The method of any one of paras 20 to 27, wherein the molar ratio of citrate to polysorbate 20 is from about 3 to about 6.

29. The method of any one of paras 20 to 28, wherein the 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.
- 5 30. The method of any one of paras 20 to 29, wherein the aqueous formulation comprises about 0.02% (w/v) polysorbate 20.
- 10 31. Use of citrate at a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7 to prevent degradation of polysorbate 20 in an aqueous formulation.
32. The use of para 31, wherein the aqueous formulation comprises sodium chloride.
33. The use of para 32, wherein the aqueous formulation is as defined in any one of paras 1 to 19.
- 15 34. The use of any one of paras 31 to 33, wherein degradation of polysorbate 20 comprises oxidation of polysorbate 20.
35. The use of any one of paras 31 to 34, wherein degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a metal ion.
- 20 36. The use of any one of paras 31 to 35, wherein degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
- 25 37. The use of para 36, 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.
- 30 38. The use of any one of paras 31 to 37, wherein the aqueous formulation is provided in a glass container, a stainless steel container or a container comprising a plastic.
- 35 39. The use of any one of paras 31 to 38, wherein the molar ratio of citrate to polysorbate 20 is from about 3 to about 6.
- 40 40. The use of any one of paras 31 to 39, wherein the 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..

41. The use of any one of paras 31 to 40, wherein the aqueous formulation comprises about 0.02% (w/v) polysorbate 20.
42. Use of the aqueous formulation of any one of paras 1 to 17 as a dilution medium for a composition.
43. The use of para 42, wherein the composition comprises a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
44. The use of para 43, 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.
45. 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 paras 1 to 17.
46. The method of para 45, wherein the composition is a pharmaceutical composition for administration.
47. The method of para 46, wherein administration is intravenous, parenteral, systemic, intracavitary, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, subconjunctival, intravitreal, intratumoral, subcutaneous, intradermal, oral or transdermal administration.
48. The composition obtained or obtainable by the method according to any one of paras 45 to 47.
49. A composition comprising polysorbate 20, citrate and sodium chloride, wherein the molar ratio of citrate to polysorbate 20 in the composition is from about 1.5 to about 7, wherein the composition is provided in lyophilised form.
50. The composition of para 49, wherein the molar ratio of citrate to polysorbate 20 in the composition is from about 3 to about 6.
51. The composition of para 49 or para 50, wherein the molar ratio of citrate to polysorbate 20 in the composition 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.
52. The composition of any one of paras 49 to 51, wherein the composition does not comprise histidine.

53. The composition of any one of paras 49 to 52, wherein the composition further comprises a metal ion.
54. The composition of any one of paras 49 to 53, further comprising a pharmaceutically-acceptable carrier, excipient or adjuvant.
55. The composition of any one of paras 49 to 54, wherein the composition is provided in a glass container, a stainless steel container or a container comprising a plastic.
56. The composition of any one of paras 49 to 55, further comprising a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
57. The composition of para 56, 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.
58. A kit comprising the aqueous formulation of any one of paras 1 to 19, or the composition of para 48.
59. A kit comprising the aqueous formulation of any one of paras 1 to 17, or the composition of any one of paras 49 to 55.
60. The kit of para 59, wherein the kit further comprises a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

The present disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject-matter described.

Aspects and embodiments of the present disclosure will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer

or step or group of integers or steps. As used herein, the term “prevent” may (also) include “reduce”. For example, in various aspects of the present invention, to “prevent”, e.g., degradation of polysorbate, means (also) to “reduce”, e.g., the degradation of polysorbate.

5 It must be noted that, as used in the specification and the appended claims, the singular forms ‘a’, ‘an’, and ‘the’ include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from ‘about’ one particular value, and/or to ‘about’ another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent ‘about’, it
10 will be understood that the particular value forms another embodiment.

As used herein, a ‘peptide’ refers to a chain of two or more amino acid monomers linked by peptide bonds. Peptides typically have a length in the region of about 2 to about 50 amino acids. A ‘polypeptide’ comprises more than one peptide bond, and comprises 3 or more amino acids.

15 Values may be expressed herein as ‘about’ a particular value. Similarly, ranges may be expressed herein as from ‘about’ a particular value, and/or to ‘about’ another particular value. The term ‘about’ in relation to a numerical value is optional, and means for example $\pm 10\%$. By way of illustration, reference e.g. to ‘about 10 %’ is to be construed as 9 % to 11 %. In instances herein where ‘about’ is recited, the value it
20 precedes is also specifically contemplated. By way of illustration, reference e.g. to ‘about 10 %’ also specifically contemplates 10 %.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the present disclosure will now be discussed
25 with reference to the accompanying figures.

Figure 1. Idealised chemical structure of PS20 (POE sorbitan monoester) and potential sites of oxidative (dark grey) and hydrolytic (light grey) degradation. $W + x + y + z = 20$, referring to the number of ethylene oxide moieties defined by the pharmacopeias.

30 **Figure 2.** Titration series of citrate to polysorbate 20 molar ratio from 0 to 15 defined an optimum range for mitigating PS20 oxidation. Formulations of 0.2 mg·ml⁻¹ PS20 in 0.9 % (w/v) NaCl with different citrate to PS20 molar ratio. The oxidative stress stability study was performed in the presence of 29 mM H₂O₂ at 40 °C / 75 % relative humidity (r.H). The PS20 content was determined by fluorescence micelle assay
35 (FMA). All values represent technical octuplets ($n = 8$) out of two replicates. The average and standard deviation (SD) values of the replicates are reported.

Figures 3A and 3B. Mitigation of oxidative PS20 degradation in diluent formulations in a long-term stability study. Formulations of 0.2 mg·ml⁻¹ PS20 in MilliQ water or in 0.9 % (w/v) NaCl in the presence
40 and absence of citrate. The citrate to PS20 molar ratio was 3.07. The samples of the oxidative long-term stability study were performed in the presence of 29 μM H₂O₂ at 40 °C / 75 % r.H. for 12 months (Figure

3A) and up to 24 months (Figure 3B). The PS20 content was determined by FMA. PS20 formulations in MilliQ water (grey bar) or 0.9 % (w/v) NaCl (white bar) in the absence of citrate. PS20 formulations in MilliQ water (grey dotted bar) or 0.9 % (w/v) NaCl (white dotted bar) in the presence of citrate. All values represent technical octuplets ($n = 8$) out of two replicates. The average and standard deviation (SD) values of the replicates are reported.

Figure 4. Oxidation marker of oxidative PS20 degradation in diluent formulations. Formulations of 0.2 mg·ml⁻¹ PS20 in 0.9 % (w/v) NaCl in the absence (A) and presence (B) citrate. The citrate to PS20 molar ratio was 3.07. The samples of the oxidative long-term stability study were performed in the presence of 29 µM H₂O₂ and stored at 40 °C / 75 % r.H. up to 12 months. The content of oxidation marker (POE5 Di, POE5 Mono, POE6 Di, POE6 Mono) was determined by a UPLC-QDA assay. (A) PS20 formulation in the absence of citrate; initial and after 3 months. (B) PS20 formulation in the presence citrate; initial and after 6 months.

Figure 5. Titration series of citrate to polysorbate 80 molar ratio from 0 to 16 defined an optimum range for mitigating PS80 oxidation. Formulations of 0.2 mg·ml⁻¹ PS80 in 0.9 % (w/v) NaCl with different citrate to PS80 molar ratio. The oxidative stress stability study was performed in the presence of 29 mM H₂O₂ at 40 °C / 75 % relative humidity (r.H). The PS80 content was determined by fluorescence micelle assay (FMA). All values represent technical octuplets ($n = 8$) out of two replicates. The average and standard deviation (SD) values of the replicates are reported.

Figures 6A and 6B. Diagram showing turbidity measurements of mAb 1 formulations (D00 to D005) in the absence (6A) and presence (6B) of citrate. The solutions were either stored in the dark or stored exposed to light. Turbidity was measured in formazin nephelometric units (FNU) using a Hach Lange instrument at a wavelength between 400-600 nm and at the following time points: 0, 9 and 24 h.

Figure 7. Table showing the results of visual inspection of mAb 1 formulations (D00 to D005), performed according to Pharmacopoeia Europea (Ph.Eur. 2.9.20). The solutions were either stored in the dark or stored exposed to light, and evaluated by visual inspection at the following time points: 0, 9 and 24 h.

Figures 8A and 8B. Diagram showing the concentration of sub-visible particles of size ≥10 µm in each mAb 1 formulation (D00 to D005), in the absence (8A) and in presence (8B) of citrate, as determined by micro-flow imaging (MFI).

Figures 9A and 9B. Diagram showing the concentration of sub-visible particles of size ≥25 µm in each mAb1 formulation (D00 to D005), in the absence (9A) and in presence (9B) of citrate, as determined by micro-flow imaging (MFI).

Examples

Example 1: Oxidative stress stability study in the presence of citrate**1.1 Study set-up**

For the preparation of the citrate-containing formulations, sodium citrate dihydrate was used. Sample solutions were filtered through 0.22-µm porosity filter cartridges (Sterivex-GV, Millipore) and 50 mL aliquots of the sterile bulk formulation were transferred into 50R borosilicate glass vials.

To enhance the oxidative stress during the stability studies, water peroxide (H₂O₂) was spiked to the PS20 samples that were stored at 40 °C. The adjusted, final H₂O₂ concentrations were 1000 ppm (29 mM) for the 8 weeks study, and 1 ppm (29 µM) for the long-term study (12 months and extended to 24 months). Vials were sealed with bromobutyl teflonized and siliconized D777-1 stoppers and closed with aluminium crimped caps. Samples were stored at 40 °C for up to 12 months, initially, and up to 24 months and analysed as described below. The containers selected for PS20-related analytics were taken randomly. Tested samples covered a citrate to PS20 molar ratio of 0.06 to 15.4 in 0.2 mg/ml PS20 and contained 0.9 % (w/v) NaCl (normal saline). 50 ml of the respective formulation was filled in 50R glass vials and sealed with a rubber stopper. Specific time point samples were analysed for the polysorbate content using the fluorescence micelle assay (FMA).

For the oxidative stress stability study, to identify the optimum in the citrate to PS20 molar ratio, the samples were spiked with H₂O₂ to a final concentration of 29 mM (1000 ppm) H₂O₂ and stored at 40 °C up to 8 weeks. A long-term stability study with a final H₂O₂ concentration of 29 µM (1 ppm), in the presence of a citrate to PS20 molar ratio of 3 was stored up to 12 months at 40 °C, initially, and extended to 24 months at 40 °C. Specific time point samples were analysed for the polysorbate content using the fluorescence micelle assay (FMA).

For the oxidative stress stability study to identify the optimum in the citrate to PS80 molar ratio, the samples were spiked with H₂O₂ to a final concentration of 29 mM (1000 ppm) H₂O₂ and stored at 40 °C up to 8 weeks.

1.2 Investigation of the effect of different molar ratios of citrate to PS20 in a formulation on degradation of PS20

An oxidative stress stability study was carried out to investigate degradation of PS20 in aqueous formulations in the absence and presence of citrate at various citrate-to-PS20 molar ratios. The formulations contained 0.2 mg·ml⁻¹ PS20 (equivalent to 0.02% w/v) and 0.9 % (w/v) NaCl, and were spiked with 29 mM H₂O₂ to induce oxidative stress.

Table 1 outlines the composition of each formulation tested. The molar concentrations are calculated based on the molecular weight (Mr) of each component (PS20 Mr = 1227 g/mol, and sodium citrate dihydrate Mr = 294.10 g/mol).

Table 1. Compositions of PS20 formulations comprising citrate used in stability study

Formulation	Citrate to PS20 molar ratio	Citrate molar concentration (μM)	PS20 molar concentration (μM)
1	0	0	163
2	0.06	10	163
3	0.31	50	163
4	0.61	100	163
5	1.54	250	163
6	3.07	500	163
7	4.61	750	163
8	6.14	1000	163
9	15.4	2500	163

The results are shown in Figure 2.

As it can be seen from Figure 2, in the absence of citrate, PS20 is degraded rapidly; after 2 weeks of storage at 40 °C, 50 % of PS20 is degraded. After 6 weeks of storage at 40 °C, the intact PS20 content decreases to below 20 % compared to the initial value.

At a citrate to PS20 molar ratio of 0.06, the presence of citrate reduces the degradation kinetics of PS20. However, even at an increased citrate to PS20 molar ratio of 0.31 after 8 weeks (at 40°C), roughly 50 % of PS20 is degraded.

In the examples described herein, a storage temperature of 40°C was chosen as additional stress factor, in addition to 29 μM H_2O_2 , which is assumed to accelerate degradation. If stability is demonstrated for storage at 40°C over a certain time, at least the same stability (over the same time) is expected for storage at lower temperatures, e.g., at about 25 °C. Usually, stability is increased (i.e., over a longer time) for storage at lower temperatures, e.g., at about 25 °C.

This result is different to the data reported by Doyle *et al.* (2019) (38), who reported that a citrate concentration of 62.5 μM , corresponding to a citrate to PS80 molar ratio of 0.4, to be sufficient to prevent polysorbate 80 (PS80) oxidation in a histidine (10 mM) formulation exposed to stainless steel. In the present study, a molar ratio of citrate to PS20 of at least 0.61 was found to be required to reduce PS20 degradation (Figure 2).

The presence of histidine buffer in the formulations tested by Doyle *et al.* (2019) (38) likely interferes with or masks the antioxidant effect of citrate since histidine has been reported to protect polysorbates against (2, 2'-azobis (2-amidinopropane) hydrochloride) (APPH)-induced oxidation, which is a model for reactive oxygen species (40).

Although PS80 and PS20 are both polysorbates, as explained hereinabove, the chemical composition of these two surfactants is different (17). In certain reports differences between the degradation sensitivity of both polysorbates upon specific stress conditions have also been described (4,42,43).

- 5 In view of the structural differences between PS80 and PS20, and the presence of histidine buffer in the formulations in Doyle *et al.* (2019) (38), the specific effect of citrate on the oxidation of PS20 cannot be reasonably translated or predicted from the study by Doyle *et al.* (2019).

10 The present study focuses on the stability of PS20 in citrate-containing formulations in the absence of histidine.

The presence of small amounts of citrate may impair protein stability (16,18,19,44–46). Gopalrathnam *et al.* (2018) conclude that the use of citrate had a negative effect on product stability: 'For the LY2951742 antibody formulation, sodium citrate buffer was not considered a viable option due to suboptimal stability performance' (47). Therefore, careful evaluation of the citrate content used to stabilise polysorbate is
15 required to ensure that the stability of any polypeptide present in the formulation is not adversely affected.

The present study finds that a citrate to PS20 molar ratio of 0.31, corresponding approximately to the conditions tested by Doyle *et al.* (2019) (38), did not prevent PS20 oxidation as 45 % of PS20 was
20 degraded after 8 weeks at 40 °C.

The inventor's citrate titration study shows surprisingly an optimum in the citrate to PS20 molar ratio required to reduce PS20 degradation. At least a citrate to PS20 molar ratio of ca. 1.5 to 3 is needed to reduce PS20 degradation. That is, the minimum citrate to PS20 molar ratio to reduce degradation is in the
25 range of ca. 1.5 to ca. 3. These data shows that about 10 times more citrate is required for the stabilisation of PS20 compared to PS80, as proposed by Doyle *et al.* (2019) (38).

As shown in Figure 2, the range of citrate to PS20 molar ratio of about 1.5 to about 6 provides increased stabilisation of PS20 over a period of up to 2 months (at least 2-fold increase with respect to non-citrate
30 containing formulations). Therefore, an optimal molar ratio of citrate to PS20 is in the range of ca. 1.5 to ca. 6, or even ca. 1.5 to ca. 7.

At a citrate to PS20 molar ratio of above ca. 7, e.g. at a ratio of 15.4 as shown in Figure 2, slightly more PS20 is degraded.

35 PS20 displayed good stability over the duration of the study (0.5, 1, 1.5 and 2 months), with a consistently low proportion of degraded PS20 for formulations comprising a range of citrate to PS20 molar ratio of ca. 1.5 to ca. 6. Within this range, the subrange of citrate to PS20 molar ratio being ca. 3 to ca. 6 produced an apparent ≥ 4 -fold increase in stability over all time points when compared with the non-citrate
40 containing formulations (Figure 2).

The highest PS20 stability was observed in formulations comprising citrate to PS20 molar ratio of 3.07 or 4.61, which showed less than 20% degradation of PS20 over 2 months at 40 °C in the presence of 29 mM H₂O₂, and no degradation of PS20 over up to 0.5 months.

- 5 In view of concerns that PS20 likely affects the stability of polypeptides at high concentrations, the citrate to PS20 molar ratio of ca. 3 (0.5 mM citrate) was selected for further investigation.

1.3 Investigation of the effect of different molar ratios of citrate to PS80 in a formulation on degradation of PS80

- 10 An oxidative stress stability study was carried out to investigate degradation of PS80 in aqueous formulations in the absence and presence of citrate at various citrate-to-PS80 molar ratios, following the procedure described in Example 1.2. The formulations contained 0.2 mg·ml⁻¹ PS80 (equivalent to 0.02% w/v) and 0.9 % (w/v) NaCl, and were spiked with 29 mM H₂O₂ to induce oxidative stress.
- 15 Table 2 outlines the composition of each formulation tested. The molar concentrations are calculated based on the molecular weight (Mr) of each component (PS80 Mr = 1310 g/mol, and sodium citrate dihydrate Mr = 294.10 g/mol).

Table 2. Compositions of PS80 formulations comprising citrate used in stability study

Formulation	Citrate to PS80 molar ratio	Citrate molar concentration (μM)	PS80 molar concentration (μM)
1	0	0	152
2	0.07	10	152
3	0.33	50	152
4	0.66	100	152
5	1.64	250	152
6	3.28	500	152
7	4.91	750	152
8	6.55	1000	152
9	16.4	2500	152

20

The results are shown in Figure 5. In the absence of citrate, PS80 is degraded rapidly, and after 4 weeks of storage at 40 °C, 100% of PS80 is degraded.

At a citrate to PS80 molar ratio of 0.33, the presence of citrate reduces the degradation kinetics of PS80.

- 25 However, after 8 weeks (at 40°C), roughly 70% of PS80 is degraded.

In the examples described herein, a storage temperature of 40°C was chosen as additional stress factor, in addition to 29 μM H₂O₂, which is assumed to accelerate degradation. If stability is demonstrated for

storage at 40 °C over a certain time, at least the same stability (over the same time) is expected for storage at lower temperatures, e.g., at about 25 °C. Usually, stability is increased (i.e., over a longer time) for storage at lower temperatures, e.g., at about 25 °C.

5 This result is different to the data reported by Doyle *et al.* (2019) (38), who reported that a citrate concentration of 62.5 µM, corresponding to a citrate to PS80 molar ratio of 0.4, to be sufficient to prevent polysorbate 80 (PS80) oxidation in a histidine (10 mM) formulation exposed to stainless steel. In the present study, a molar ratio of citrate to PS80 of at least 0.66 was found to be required to reduce PS80 degradation to a considerable extent (Figure 5).

10

The present study finds that a citrate to PS80 molar ratio of 0.33, corresponding approximately to the conditions tested by Doyle *et al.* (2019) (38), did not prevent PS80 oxidation as 70% of PS80 was degraded after 8 weeks at 40 °C.

15 The inventors' citrate titration study shows surprisingly an optimum in the citrate to PS80 molar ratio required to reduce PS80 degradation. At least a citrate to PS80 molar ratio of ca. 1.6 to 3.2 is needed to reduce PS80 degradation. That is, the minimum citrate to PS80 molar ratio to reduce degradation is in the range of ca. 1.6 to ca. 3.2. This data shows that about 4 times more citrate is required for the stabilisation of PS80 compared to the amount proposed by Doyle *et al.* (2019) (38).

20

As shown in Figure 5, the range of citrate to PS80 molar ratio of about 1.6 to about 6.5 provides increased stabilisation of PS80 over a period of up to 2 months (at least 2-fold increase with respect to non-citrate-containing formulations). Therefore, an optimal molar ratio of citrate to PS80 is in the range of ca. 1.6 to ca. 6.5, or even ca. 1.6 to ca. 7.

25

Moreover, the present data show that at a citrate to PS80 molar ratio of above ca. 7, e.g. at a ratio of 16.4 as shown in Figure 5, slightly more PS80 is degraded. Doyle *et al.* (2019) fail to identify this trend.

30 PS80 displayed good stability over the duration of the study (1, 1.5 and 2 months), with a consistently low proportion of degraded PS80 for formulations comprising a range of citrate to PS80 molar ratio of ca. 1.6 to ca. 6.5. Within this range, the subrange of citrate to PS80 molar ratio being ca. 3 to ca. 6 stabilises more than 65% of PS80, while the non-citrate-containing samples contain 0% PS80 (Figure 5).

35 The highest PS80 stability was observed in formulations comprising citrate to PS80 molar ratio of 3.28 or 4.91, which showed less than 33% degradation of PS80 over 2 months at 40 °C in the presence of 29 mM H₂O₂, and about 20% degradation of PS80 over up to 1 month. The formulations comprising a citrate to PS80 molar ratio of 6.55 also showed less than 33% degradation of PS80 observed over 2 months.

40 The present results show that stabilisation of PS80 in sodium chloride containing formulations, and even in the absence of histidine buffer, is achieved at molar ratios of citrate to PS80 of ca. 1.6 to ca. 7, with the optimal range being from ca. 3 to ca. 6.

Example 2: Citrate provides long-term protection against oxidative degradation of PS20

2.1 Long term stability study of PS20 formulations comprising citrate

5 As a citrate to PS20 molar ratio of ca. 3 is the lowest amount necessary to reduce PS20 degradation as shown in Figure 2, the stability studies were extended using 0.2 mg·ml⁻¹ PS20 in MilliQ water and 0.9 % (w/v) NaCl, in the presence and absence of this citrate to PS20 molar ratio.

10 The presence of sodium chloride upon PS20 stability is also evaluated. Thus, an oxidative long-term stability study was performed to monitor whether a citrate to PS20 molar ratio of 3 is sufficient to stabilise PS20 for up to 12 months, and further for up to 24 months.

15 Figures 3A and 3B demonstrate the results of the long-term stability study. It is obvious, that for both formulations in the absence of citrate, already after 3 months at 40 °C, the PS20 content dropped below 0.025 mg·ml⁻¹ PS20.

20 The two samples formulated in the presence of citrate showed PS20 stabilisation for up to 12 months storage at 40 °C (Figure 3A). Thus, a citrate to PS20 molar ratio of ca. 3 is efficient in preventing PS20 degradation for a period of at least 12 months. A slight decrease of about 10 % is noted, and the inventors expect that this is due to different degradation pathways, as reported by Dwivedi *et al.* (17). The presence of sodium chloride has no relevant impact on PS20 stability.

25 Significant stabilisation of PS20 is maintained for even up to 24 months of storage at 40 °C, as shown in Figure 3B. The concentration of PS20 is maintained well above 0.1-0.15 mg·ml⁻¹ in the citrate-containing samples after 24 months of storage at 40 °C, while in the absence of citrate, there are practically no detectable levels of PS20.

2.2 Investigation of markers of oxidative degradation of PS20

30 Specific markers for oxidative degradation of PS20 were investigated in order to get further insight into the degradation mechanisms occurring in aqueous formulations of PS20. Table 3 shows relevant oxidation products of PS20 which are indicative of oxidative degradation.

35 **Table 3.** Oxidation markers of PS20

PS20 oxidation marker description and abbreviation	Structure
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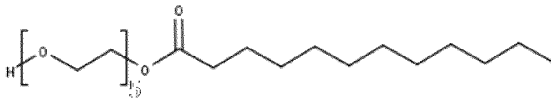
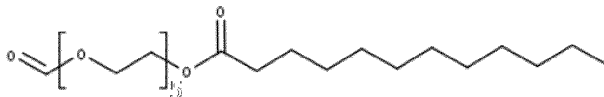
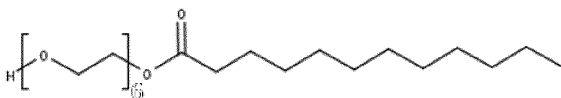
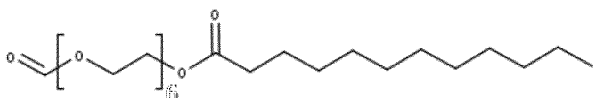
Mono-ester of laurate with 5 POE subunits. POE5 Mono	
Di-ester of formate and laurate with 5 POE subunits. POE5 Di	
Mono-ester of laurate with 6 POE subunits. POE6 Mono	
Di-ester of formate and laurate with 6 POE subunits. POE6 Di	

Figure 4 demonstrates the levels of oxidation markers (POE5 Di, POE5 Mono, POE6 Di, POE6 Mono) detected in formulations containing PS20 and citrate at a molar ratio of citrate to PS20 of 3.07, or no citrate. The formulation containing PS20 in the absence of citrate showed enormous oxidation of PS20, as evidenced by the levels of markers already after 3 months at 40 °C. The citrate-containing samples showed no increased level of oxidation markers as exemplarily shown at the graph depicting the levels after 6 months/40 °C. This observation is consistent with the results shown in Figure 3.

The inventors have identified a range of citrate to PS20 molar ratios that provide long-term protection of PS20 against oxidative degradation.

2.3 Conclusion

The stability experiments of PS20-containing formulations described herein involving a titration oxidative stability study at 40 °C identified an optimum citrate to PS20 molar ratio at approx. 3 to 7. Within this range, degradation of PS20 can be hampered. When tested in formulations containing another polysorbate (polysorbate 80), an equivalent observation was made by the inventors. The inventors have identified an optimum citrate to PS molar ratio at approximately 3 to 7, to stabilise polysorbate in an aqueous formulation.

The inventors further evaluated the minimal citrate to PS20 molar ratio of ca. 3 that is required to stabilise PS20 in a long-term oxidative stability study for up to 12, and further up to 24 months under extreme oxidising conditions (29 µM H₂O₂, at 40 °C). No relevant PS20 degradation is observed in a formulation containing 0.2 mg·ml⁻¹ in 0.9 % (w/v) NaCl at a citrate to PS20 molar ratio of ca. 3 up to 12 months at 40

°C under these oxidative conditions, and minimal degradation of PS20 is observed under the same conditions even after 24 months.

This finding is particularly useful as the presence of citrate may induce formation of protein particles and/or gelation during the processing and formulation of biologics (16,18,19,44–46). Even at buffer concentrations as low as 5 mM citrate, reported case studies have described increased aggregation rates of proteins in citrate-containing formulations (46). A citrate to PS (e.g. PS20) molar ratio of ca. 3 translates to a concentration of citrate in the range of 0.5 mM in a formulation which contains 0.2 mg·ml⁻¹ PS20, which is ten times lower than 5 mM.

The present results show that stabilisation of polysorbate (PS20, PS80 etc.) in sodium chloride containing formulations, and even in the absence of histidine buffer, is achieved at molar ratios of citrate to PS20 or citrate to PS80 of ca. 1.5 to ca. 7, with the optimal range being from ca. 3 to ca. 6.

Example 3: Materials and Methods used in Examples 1-2

3.1 Materials and Reagents

Acetonitrile (ACN) for liquid chromatography, hydrogen peroxide 35 % (w/v) (H₂O₂), ammonium ferrous (II) sulfate hexahydrate ((NH₄)₂Fe(SO₄) x 6H₂O) and xylene orange disodium salt (XODS) were obtained from Carl Roth GmbH (Karlsruhe, Germany). Ammonium formate (NH₄HCO₃), 30 % (w/v) Brij-35, N-phenyl-1-naphthylamine (NPN), iron chloride (FeCl₂ and FeCl₃) was gained from Sigma-Aldrich (St. Louis, MO, USA). Formic acid, sulfuric acid (H₂SO₄) and sodium chloride (NaCl) were obtained from Merck KGaA (Darmstadt, Germany). Sodium citrate dihydrate was obtained from Jungbunzlauer GmbH (Pernhofen, Austria). Methanol (MeOH) of LC-MS grade was purchased from Honeywell International Inc. (Charlotte, NC, USA). Trometamol (Tris) was obtained from Angus Chemie GmbH (Ibbenbüren, Germany). Polysorbate 20 of high purity grade (PS20 HP) was obtained from Croda (Arnhem, Netherlands). Neat PS20 HP material was stored protected from light at 2-8 °C under nitrogen overlay. High purified water was produced by using a Milli-Q IQ 7000 system from Merck KGaA (Darmstadt, Germany) and is named 'water' in the following. 50 mL Fiolax[®] vials (glass type I) were purchased from Schott AG (Mainz, Germany). 20 mm B2-coated FluroTec[™] (D777-1) stoppers were procured from West Pharmaceutical Services (Eschweiler, Germany). Polysorbate 80 of high purity grade (PS80 HP) was obtained from Croda (Arnhem, Netherlands).

3.2 Methods

Fluorescence Micelle Assay. The fluorescence micelle assay (FMA) was adapted from Lippold *et al.* (2017) (41). The hydrophobic fluorophore NPN was used to quantify the concentration of PS20 by partitioning into PS micelles (48). The PS20 HP concentrations in the testing solutions were quantitated utilizing a Fluent Automation Workstation from Tecan Group AG (Männedorf, Switzerland). Therefore, 240 µl of the FMA buffer consisting of 5 µM NPN, 0.0015 % (w/v) Brij-35, 150 mM NaCl, 5 % (v/v) ACN and 50 mM Tris at pH 8.0 was added to 10 µl PS-containing samples and incubated at 35 °C for 1 min at 167 rpm. Technical replicates of four (*n* = 4) were measured. The sample fluorescence was detected with

a fluorescence plate reader (Infinite M200pro Tecan Group AG, Männedorf, Switzerland) with an excitation wavelength of 350 nm and an emission wavelength of 420 nm. The PS20 concentration was obtained by using standard calibration samples of PS20 (0, 0.1, 0.3, 0.6 mg·ml⁻¹). Limit of detection (LOD) was 0.023 mg·ml⁻¹ PS20 and the limit of quantification (LOQ) was 0.069 mg·ml⁻¹. The PS80 concentration was obtained by using standard calibration samples of PS80 (0, 0.05, 0.2, 0.3 mg·ml⁻¹). limit of quantification (LOQ) was 0.05 mg·ml⁻¹.

Polysorbate 20 oxidation markers analytics. The characterization of the PS20 oxidation marker (see Table 3) was performed using a UPLC assay coupled to a Qda mass detector, essentially according to the method described in Birdsall *et al.*, 'Quantitative Analysis of Polysorbate 20/80 in Protein-Based Biopharmaceuticals Using A One-Pot RPLC-MS Based Platform Method' Waters Corporation, GlaxoSmithKline; Application Note 720007249, May 2021.

Example 4: Investigation of the effect of a citrate-containing formulation as dilution media for a protein

As described in Example 2, citrate-containing formulations according to the invention exhibit stabilisation of polysorbate. In view of studies (47) reporting that high levels citrate negatively affect the stability of polypeptides (e.g. antibodies) in formulations, the inventors further investigated the physical stability of protein samples prepared using the citrate-polysorbate formulations of the present invention as dilution media.

A monoclonal antibody (mAb 1) was diluted into an aqueous solution of 0.9 % (w/v) NaCl and 0.2 mg/mL polysorbate 20 in the absence of citrate (first dilution medium) or in the presence of 0.5 mM citrate (second dilution medium).

By employing serial dilutions using the specified dilution media, the concentration of the antibody varied in each sample. The protein was diluted to generate the following samples: D0: no dilution, D01: 5.0 mg/mL, D02: 1.0 mg/mL, D03: 0.1 mg/mL, D04: 0.01 mg/mL, D05: 0.00001 mg/mL of mAb 1. Table 4 outlines the composition of each antibody formulation sample. The samples that contain citrate have a citrate to PS20 molar ratio of 3.07.

Table 4. The composition of samples D00 to D05

Sample name	mAb 1 concentration (mg·ml ⁻¹)	NaCl concentration (% w/v)	PS20 concentration (mg·ml ⁻¹)	Citrate concentration (mM)
D00	10	0.9	0.2	0.5
				-
D01	5	0.9	0.2	0.5
				-
D02	1	0.9	0.2	0.5
				-

D03	0.1	0.9	0.2	0.5
				-
D04	0.01	0.9	0.2	0.5
				-
D05	1 x 10 ⁻⁵	0.9	0.2	0.5
				-

The solutions were evaluated by visual inspection, turbidity measurements and sub-visible particle (microparticle) concentration measurements at the following time points: 0, 9 and 24 h. Two different storage conditions were tested: storage in the dark, and storage while exposed to light.

5

Figures 6A and 6B demonstrate that the presence a low concentration of citrate in the antibody formulation does not significantly affect the turbidity of the antibody solutions at various time points, and even at high antibody concentrations, as compared with the turbidity of samples not containing citrate.

- 10 Visual inspection of the samples performed according to Pharmacopoeia Europea (Ph.Eur. 2.9.20) further confirms that the presence of a low concentration of citrate does not lead to visible particle formation in the samples (Figures 7A and 7B).

- 15 Microflow Imaging (MFI) was used to determine sub-visible particle formation. All citrate-containing samples contain a very low concentration of microparticles of $\geq 10 \mu\text{m}$ (Figure 8B) and of microparticles of $\geq 25 \mu\text{m}$ (Figure 9B), the concentration being either lower or comparable to that measured in the corresponding samples which do not contain citrate (Figures 8A and 9A, respectively).

- 20 These results show that a concentration of citrate and/or a molar ratio of citrate to PS according to the present invention prevents degradation of PS without inducing an increase in turbidity, without negatively affecting the photostability of the protein, and without inducing particle formation in protein samples over time.

25 References

A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for some of these references are provided below. The entirety of each of these references is incorporated herein.

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Claims:

1. An aqueous formulation comprising polysorbate, citrate and sodium chloride.
- 5 2. The aqueous formulation of claim 1, wherein the concentration of citrate in the aqueous formulation is from about 50 μ M to about 2.5 mM.
3. The aqueous formulation of claim 1 or claim 2, wherein the concentration of citrate in the aqueous formulation is from about 250 μ M to about 1 mM.
- 10 4. The aqueous formulation of any one of claims 1 to 3, wherein the concentration of polysorbate in the aqueous formulation is about 0.02% (w/v).
5. The aqueous formulation of any one of claims 1 to 4, wherein the concentration of sodium
15 chloride in the aqueous formulation is about 0.9 % (w/v).
6. The aqueous formulation of any one of claims 1 to 5, wherein the molar ratio of citrate to polysorbate in the aqueous formulation is from about 1.5 to about 7.
- 20 7. The aqueous formulation of any one of claims 1 to 6, wherein the molar ratio of citrate to polysorbate is from about 3 to about 6.
8. The aqueous formulation of any one of claims 1 to 7, wherein the 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
25 3.8, or about 3.9.
9. The aqueous formulation of any one of claims 1 to 8, wherein the polysorbate is polysorbate 20 or polysorbate 80, preferably wherein the polysorbate is polysorbate 20.
- 30 10. The aqueous formulation of any one of claims 1 to 9, wherein the aqueous formulation does not comprise histidine.
11. The aqueous formulation of any one of claims 1 to 10, wherein the aqueous formulation further comprises a metal ion.
- 35 12. The aqueous formulation according to any one of claims 1 to 11, wherein the concentration of sodium chloride in the aqueous formulation is about 0.9 % (w/v), wherein the polysorbate is polysorbate 80, and wherein the molar ratio of citrate to polysorbate in the aqueous formulation is from about 3 to about 6.

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13. The aqueous formulation according to any one of claims 1 to 12, wherein the aqueous formulation consists essentially of polysorbate, citrate, water and sodium chloride.
14. The aqueous formulation according to any one of claims 1 to 13, wherein the aqueous formulation consists essentially of:
- about 0.02% (w/v) polysorbate,
 - about 0.9 % (w/v) sodium chloride,
 - water, and
 - 0.25 mM to 1 mM citrate.
15. The aqueous formulation according to claim 14, wherein the aqueous formulation consists essentially of:
- about 0.02% (w/v) polysorbate,
 - about 0.9 % (w/v) sodium chloride,
 - water, and
 - about 0.5 mM citrate.
16. An aqueous formulation comprising polysorbate 20, citrate and sodium chloride.
17. The aqueous formulation of claim 16, wherein the concentration of citrate in the aqueous formulation is from about 50 μ M to about 2.5 mM.
18. The aqueous formulation of claim 16 or claim 17, wherein the concentration of citrate in the aqueous formulation is from about 250 μ M to about 1 mM.
19. The aqueous formulation of any one of claims 16 to 18, wherein the concentration of polysorbate 20 in the aqueous formulation is about 0.02% (w/v).
20. The aqueous formulation of any one of claims 16 to 19, wherein the concentration of sodium chloride in the aqueous formulation is about 0.9 % (w/v).
21. The aqueous formulation of any one of claims 16 to 20, wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 1.5 to about 7.
22. The aqueous formulation of any one of claims 16 to 21, wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 3 to about 6.
23. The aqueous formulation of any one of claims 16 to 22, wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation 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.

24. The aqueous formulation of any one of claims 16 to 23, wherein the aqueous formulation does not comprise histidine.
25. The aqueous formulation of any one of claims 16 to 24, wherein the aqueous formulation further comprises a metal ion.
26. The aqueous formulation according to any one of claims 16 to 25, wherein the concentration of sodium chloride in the aqueous formulation is about 0.9 % (w/v), and wherein the molar ratio of citrate to polysorbate 20 in the aqueous formulation is from about 3 to about 6.
27. The aqueous formulation according to any one of claims 16 to 26, wherein the aqueous formulation consists essentially of polysorbate 20, citrate, water and sodium chloride.
28. The aqueous formulation according to any one of claims 16 to 27, wherein the aqueous formulation consists essentially of:
- about 0.02% (w/v) polysorbate 20 (PS20),
 - about 0.9 % (w/v) sodium chloride,
 - water, and
 - 0.25 mM to 1 mM citrate.
29. The aqueous formulation according to claim 28, wherein the aqueous formulation consists essentially of:
- about 0.02% (w/v) polysorbate 20 (PS20),
 - about 0.9 % (w/v) sodium chloride,
 - water, and
 - about 0.5 mM citrate.
30. The aqueous formulation of any one of claims 1 to 29, 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.
31. The aqueous formulation of any one of claims 1 to 30, further comprising a pharmaceutically-acceptable carrier, excipient or adjuvant.
32. The aqueous formulation of any one of claims 1 to 31, wherein the aqueous formulation is provided in a glass container, a stainless steel container or a container comprising a plastic.
33. The aqueous formulation of any one of claims 1 to 32, further comprising a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

34. The aqueous formulation of claim 33, 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.
35. A method for preventing degradation of polysorbate in an aqueous formulation, the method comprising adding citrate at a final molar ratio of citrate to the polysorbate of about 1.5 to about 7.
36. Use of citrate at a final molar ratio of citrate to polysorbate of about 1.5 to about 7 to prevent degradation of the polysorbate in an aqueous formulation.
37. The method of claim 35 or the use according to claim 36, wherein the polysorbate is polysorbate 20 or polysorbate 80, preferably wherein the polysorbate is polysorbate 20.
38. The method of claim 35 or 37, or the use according to claim 36 or 37, wherein the aqueous formulation comprises sodium chloride.
39. The method of any one of claims 35, 37 and 38, or the use according to any one of claims 36, 37, and 38, wherein the aqueous formulation is an aqueous formulation according to any one of claims 1 to 15.
40. The method of any one of claims 35 and 37 to 39, or the use according to any one of claims 36 to 39, wherein degradation of the polysorbate comprises oxidation of the polysorbate.
41. The method of any one of claims 35 and 37 to 40, or the use according to any one of claims 36 to 40, wherein degradation of the polysorbate comprises degradation of the polysorbate in the presence of a metal ion.
42. The method of any one of claims 35 and 37 to 41, or the use according to any one of claims 36 to 41, wherein degradation of the polysorbate comprises degradation of the polysorbate in the presence of a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
43. The method or the use according to claim 42, 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.

44. The method of any one of claims 35 and 37 to 43, or the use according to any one of claims 36 to 43, wherein the aqueous formulation is provided in a glass container, a stainless steel container or a container comprising a plastic.
- 5 45. The method of any one of claims 35 and 37 to 44, or the use according to any one of claims 36 to 44, wherein the molar ratio of citrate to polysorbate is from about 3 to about 6.
46. The method of any one of claims 35 and 37 to 45, or the use according to any one of claims 36 to 45, wherein the molar ratio of citrate to polysorbate is about 3, about 3.1, about 3.2, about 3.3, about 3.4,
10 about 3.5, about 3.6, about 3.7, about 3.8, or about 3.9.
47. The method of any one of claims 35 and 37 to 46, or the use according to any one of claims 36 to 46, wherein the aqueous formulation comprises about 0.02% (w/v) polysorbate.
- 15 48. A method for preventing degradation of polysorbate 20 in an aqueous formulation, the method comprising adding citrate at a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7.
49. Use of citrate at a final molar ratio of citrate to polysorbate 20 of about 1.5 to about 7 to prevent degradation of polysorbate 20 in an aqueous formulation.
- 20 50. The method of claim 48, or the use according to claim 49, wherein the aqueous formulation comprises sodium chloride.
51. The method of claim 48 or 50, or the use according to claims 49 or 50, wherein the aqueous
25 formulation is an aqueous formulation according to any one of claims 16 to 34.
52. The method of any one of claims 48 and 50 to 51, or the use according to any one of claims 49 to 51, wherein degradation of polysorbate 20 comprises oxidation of polysorbate 20.
- 30 53. The method of any one of claims 48 and 50 to 52, or the use according to any one of claims 49 to 52, wherein degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a metal ion.
54. The method of any one of claims 48 and 50 to 53, or the use according to any one of claims 49 to
35 53, wherein degradation of polysorbate 20 comprises degradation of polysorbate 20 in the presence of a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.
55. The method or the use according to claim 54, wherein the peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis is selected from: an antigen-binding peptide/polypeptide, an antigen-binding
40 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.

56. The method of any one of claims 48 and 50 to 55, or the use according to any one of claims 49 to 55, wherein the aqueous formulation is provided in a glass container, a stainless steel container or a container comprising a plastic.

57. The method of any one of claims 48 and 50 to 56, or the use according to any one of claims 49 to 56, wherein the molar ratio of citrate to polysorbate 20 is from about 3 to about 6.

58. The method of any one of claims 48 and 50 to 57, or the use according to any one of claims 49 to 57, wherein the 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.

59. The method of any one of claims 48 and 50 to 58, or the use according to any one of claims 49 to 58, wherein the aqueous formulation comprises about 0.02% (w/v) polysorbate 20.

60. Use of the aqueous formulation of any one of claims 1 to 34 as a dilution medium for a composition.

61. The use of claim 60, wherein the composition comprises a peptide/polypeptide for use in therapy, prophylaxis and/or diagnosis.

62. The use of claim 61, 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.

63. 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 34.

64. The method of claim 63, wherein the composition is a pharmaceutical composition for administration.

65. The method of claim 64, wherein administration is intravenous, parenteral, systemic, intracavitary, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, subconjunctival, intravitreal, intratumoral, subcutaneous, intradermal, oral or transdermal administration.

66. The composition obtained or obtainable by the method according to any one of claims 63 to 65.