upstream processes. Downstream purification processes (e.g., Protein A) are capable of removal of HCPs; however, it has been shown that some HCPs can be co-purified with API molecules that have similar properties and are thus retained in trace quantities in the drug substance and drug product (K. Lee, et al., A Chinese Hamster Ovary Cell Host Cell Protein That Impacts PS-80 Degradation. AccBio Conference (2015). Presumably, lipases with high activity can result in significant polysorbate degradation even at undetectable levels. There are numerous ongoing efforts to identify and remove lipases from protein drugs by engineering cells with reduced lipase expression and via downstream processing steps (e.g., chromatography). However, the enzymatic degradation of PS20 and PS80 remains a significant challenge in biopharmaceutical development and there have been no significant efforts reported to identify optimal formulations for reducing hydrolytic or catalytic PS20 degradation.

[0009] Polysorbate degradation has numerous consequences that may impact the stability and shelf-life of protein drug formulations. Polysorbate degradants include poorly soluble fatty acids that may result in the formation of visible and subvisible particles in the solution. The loss of PS20 may also reduce the protective effects of PS20 for protein formulations. Additionally, a spiking study demonstrated that some of the PS20-related degradants can impact stability of protein drugs; however, no impact was observed under pharmaceutically relevant conditions (Kishore et al., *Pharm. Res.* 28:1194-1210 (2011).

[0010] What is needed is a method of reducing polysorbate degradation so that the protective effects of polysorbate on formulations (e.g., polypeptides) are maintained over time. This will result in more stable polypeptide formulations during processing, long-term storage, and during administration which in turn will lengthen the shelf life of polypeptide formulations and reduce waste caused by degraded and expired formulations.

[0011] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0012] In one aspect, the present disclosure refers to a method of reducing enzymatic polysorbate 20 degradation in an aqueous formulation comprising polysorbate 20, the method comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1; wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20. In some examples, the present disclosure refers to a method of reducing polysorbate degradation in an aqueous formulation comprising a polysorbate, the method comprising adding a cyclodextrin to the formulation wherein the resulting w/w ratio of cyclodextrin to

polysorbate is greater than about 37.5:1, wherein the formulation comprises about 0.005%-0.4% polysorbate. In some aspects, the present disclosure refers to a method of reducing enzymatic polysorbate 20 degradation in an aqueous formulation comprising polysorbate 20, the method comprising adding a cyclodextrin to the formulation to a concentration of 0.01%-30%, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1, wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20. In some aspects, the present disclosure refers to a method of reducing the amount of sub-visible and visible particles caused by enzymatic polysorbate 20 degradation in an aqueous formulation comprising polysorbate 20, comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1; wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20. In some examples, the disclosure provides a method of reducing the amount of sub-visible and visible particles in an aqueous formulation comprising polysorbate, comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate is greater than about 37.5:1, wherein the formulation comprises polysorbate and a polypeptide. In some aspects, the present disclosure refers to a method to disaggregate and solubilize enzymatic polysorbate 20 degradation products in an aqueous formulation comprising polysorbate 20, the method comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1; wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20. In some examples the disclosure provides a method to disaggregate and solubilize polysorbate degradation products in an aqueous formulation comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate is greater than about 37.5:1, wherein the formulation comprises polysorbate and a polypeptide.

[0013] In some examples of the above aspects, the polysorbate is polysorbate 20 or polysorbate 80. In some examples, the cyclodextrin is HP-β cyclodextrin, HP-γ cyclodextrin, or sulfobutyl ether β-cyclodextrin. In some examples, the concentration of polysorbate in the formulation is in the range of about 0.01% to 0.4%. In some examples, the concentration of polysorbate in the formulation is in the range of about 0.01% to 0.1%. In some examples, the concentration of polysorbate in the formulation is about 0.02%. In some examples, the concentration of cyclodextrin in the formulation is in the range of about 0.5-30%. In some examples, the concentration of cyclodextrin in the formulation is about 15%.

[0014] In some examples of the above aspects and examples, the polysorbate degradation is reduced by about 50%, about 75%, about 80%, about 85%, about 90%, about 95% or about 99%. In some examples, less than about 1,000, about 750, about 500, about 250, about 150, about 100, about 50, or about 25 polysorbate particles greater than about 2 microns in diameter/mL are formed.

[0015] In some examples of the above aspects and examples, the formulation comprises a polypeptide. In some examples, the polypeptide is an antibody. In some examples, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody or antibody fragment. In some examples, the polypeptide concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of reducing enzymatic polysorbate 20 degradation in an aqueous formulation comprising polysorbate 20, the method comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1; wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20.

2. A method of reducing enzymatic polysorbate 20 degradation in an aqueous formulation comprising polysorbate 20, the method comprising adding a cyclodextrin to the formulation to a concentration of 0.01%-30%, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1, wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20.

3. A method of reducing the amount of sub-visible and visible particles caused by enzymatic polysorbate 20 degradation in an aqueous formulation comprising polysorbate 20, comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1; wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20.

4. A method to disaggregate and solubilize enzymatic polysorbate 20 degradation products in an aqueous formulation comprising polysorbate 20, the method comprising adding a cyclodextrin to the formulation, wherein the resulting w/w ratio of cyclodextrin to polysorbate 20 is greater than 37.5:1 and less than 67:1; wherein the formulation comprises 0.001% to less than 0.01% polysorbate 20.

5. The method of any one of claims 1-4, wherein the cyclodextrin is HP-β cyclodextrin, HP-γ cyclodextrin, or sulfobutyl ether β-cyclodextrin.

6. The method of any one of claims 1-5, wherein the concentration of cyclodextrin in the formulation is in the range of 0.5 to 30%.

7. The method of any one of claims 1-6, wherein the concentration of cyclodextrin in the formulation is about 15%.

8. The method of any one of claims 1-7, wherein the enzymatic polysorbate 20 degradation is reduced by about 50%, about 75%, about 80%, about 85%, about 90%, about 95% or about 99%.

9. The method of any one of claims 1-8, wherein less than 1,000, 750, 500, 250, 150, 100, 50, or 25 polysorbate particles greater than 2 microns in diameter/mL are formed.

10. The method of any one of claims 1-9, wherein the formulation is stable at 2 °C to 8 °C for at least six months, at least 12 months, at least 18 months, or at least 24 months.

11. The method of any one of claims 1-10, wherein the formulation is stable at 1°C to 10 °C for at least forty-eight months.

12. The method of any one of claims 1-11, wherein the formulation is stable at 2 °C to 8 °C for at least forty-eight months.

13. The method of any one of claims 1-12, wherein the formulation further comprises a polypeptide.

14. The method of claim 13, wherein the polypeptide is an antibody.

15. The method of claim 14, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody or an antibody fragment.

16. The method of any one of claims 13-15, wherein the polypeptide concentration in the formulation is 1 mg/mL to 250 mg/mL.

17. The method of any one of claims 1-16, wherein the formulation has a pH of 4.5 to 7.0.

18. The method of any one of claims 1-17, wherein the formulation has a pH of 4.5 to 6.0.

19. The method of any one of claims 1-18, wherein the formulation has a pH of about 6.0.

20. The method of any one of claims 1-19, wherein the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

21. The method of any one of claims 1-20, wherein the formulation is a pharmaceutical formulation suitable for administration to a subject.

22. The method of any one of claims 1-21, wherein the formulation is pharmaceutical formulation suitable for intravenous, subcutaneous, intramuscular, or intravitreal administration to a subject.

23. The method of claim 14, wherein the antibody is produced recombinantly.

24. The method of claim 23, wherein the antibody is produced in Chinese hamster ovary cells.