SUMMARY

[0008] The present disclosure refers to a method of reducing oxidation of an antibody in an aqueous formulation comprising adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the antibody, wherein the antibody comprises at least one tryptophan residue with a solvent-accessible surface area (SASA) of greater than about 80Å^2. The present disclosure also refers to a method of reducing oxidation of an antibody in an aqueous formulation comprising adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the antibody, wherein the antibody comprises at least one tryptophan residue with a solvent-accessible surface area (SASA) of greater than about 30%. In a one aspect, the present disclosure refers to a method of reducing oxidation of an antibody in an aqueous formulation comprising determining the SASA values of tryptophan residues in the antibody and adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the antibody if at least one tryptophan residue has a solvent-accessible surface area (SASA) of greater than 80Å^2. In some examples, the SASA value of the tryptophan residues in calculated by molecular dynamic simulation.

[0009] In some examples, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM. In some examples, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM. In some examples, the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM.

[0010] In some examples, the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%. In some examples, the formulation is stable at about 2 °C to about 8 °C for about 1095 days.

[0011] In some examples, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL. In some examples, the formulation has a pH of about 4.5 to about 7.0. In some examples, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

[0012] In some examples, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some examples, the protein 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 an antibody fragment.

[0013] Further provided is a liquid formulation comprising an antibody and an amount of N-acetyltryptophan to prevent oxidation of the antibody,

80Å2, is subject to oxidation, adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide, adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition, incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C, measuring the polypeptide for oxidation of tryptophan residues in the polypeptide, wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

[0018] In some examples of the above aspects, the SASA value of the tryptophan residues in calculated by molecular dynamic simulation.

[0019] The present disclosure refers to a kit comprising the liquid formulation as disclosed herein. The present disclosure also refers to an article of manufacture comprising the liquid formulation as disclosed herein.

[0020] Provided herein are formulations comprising a protein and N-acetyl-tryptophan (NAT), and methods of making and using the formulations.

[0021] In some examples, the liquid formulation is a pharmaceutical formulation suitable for administration to a subject. In some examples, the formulation is aqueous.

[0022] In some examples, the NAT prevents oxidation of tryptophan in the protein.

[0023] In some examples, the protein in the formulation is susceptible to oxidation. In some examples, tryptophan in the protein is susceptible to oxidation. In some examples, the protein is an antibody (e.g., a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment). In some examples, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

[0024] In some examples, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent. In some examples, the formulation has a pH of about 4.5 to about 7.0.

[0025] The present disclosure refers to a method to determine if a polypeptide in a liquid formulation comprises a tryptophan residue susceptible to oxidation, the method comprising calculating one or more molecule descriptors based on the amino acid sequence of the polypeptide for each tryptophan residue in the polypeptide and applying the one or more molecule descriptors to a machine learning algorithm trained on the one or more molecule descriptors to predict tryptophan oxidation, wherein the molecule descriptors include one or

CLAIMS

What is claimed is:

1. A method of reducing oxidation of an antibody in an aqueous formulation comprising determining the SASA values of tryptophan residues in the antibody and adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the antibody if at least one tryptophan residue has a solvent-accessible surface area (SASA) of greater than 80$Å^2$.

2. The method of claim 1, wherein the SASA value of the tryptophan residues is calculated by molecular dynamic simulation.

3. The method of any one of claims 1 or 2, wherein the N-acetyltryptophan is added to the formulation to a concentration of 0.1 mM to 5 mM.

4. The method of claim 3, wherein the N-acetyltryptophan is added to the formulation to a concentration of 0.1 mM to 1 mM.

5. The method of claim 4, wherein the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM.

6. The method of any one of claims 1-5, wherein the oxidation of the antibody is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%.

7. The method of any one of claims 1-6, wherein the formulation is stable at 2 °C to 8 °C for about 1095 days.

8. The method of any one of claims 1-7, wherein the antibody concentration in the formulation is 1 mg/mL to 250 mg/mL.

9. The method of any one of claims 1-8, wherein the formulation has a pH of 4.5 to 7.0.

10. The method of any one of claims 1-9, 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.

11. The method of any one of claims 1-10, wherein the formulation is a pharmaceutical formulation suitable for administration to a subject.

12. The method of any one of claims 1-11, 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.