formulation conditions. On the contrary, recent studies have unveiled difficult-to-remove CHO host cell protein (HCP) impurities, which are thought to be involved in the hydrolysis of polysorbate in biopharmaceutical formulations. It has been reported that the presence of putative phospholipase B-like 2 (PLBL2) and lipoprotein lipase (LPL) or other types of lipases and esterases may attribute to polysorbate degradation [22-24].

Recent findings establish that lipases associated with biologics manufacturing are expressed in upstream processes. In general, downstream purification processes (e.g., Protein A) are capable of removal of HCPs: however, it has been shown that some HCPs can be difficult to remove due to specific interactions with antibodies (Vanderlaan, M., et al., Bioproc Int. 2015, 13:18–29) 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)). For storage of biopharmaceutical products, it is therefore important to control and minimize the degradation of polysorbate and the formation of particles which are for example formed by accumulation of free fatty acids. In particular, the enzymatic degradation of polysorbate needs to be addressed.

There are ongoing efforts for example to identify and remove lipases from protein drugs e.g. by engineering cells with reduced lipase expression (WO2015/095568) and efforts to find formulations with reduced polysorbate degradation (WO2017/117311) to address the problem. However, there is still a need for controlling/reducing the enzymatic degradation of surfactants like polysorbate and it remains a significant challenge in biopharmaceutical development.

In WO2016/057739 a process for reducing subvisible particles in a pharmaceutical formulation is presented.

Summary

Herein is reported a method for the purification of a protein or polypeptide (e.g. an antibody) using a polysorbate in a purification step, for example in a chromatographic step, to reduce the hydrolytic activity in a sample or preparation. Also reported are methods for producing proteins or polypeptides and protein or polypeptide preparations.

Also reported herein is a method for the purification of a protein or polypeptide (e.g. an antibody) using a polysorbate in a purification step, for example in a

ii)

a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,

b) applying the mixture of step a) to a chromatography material, and

c) recovering the protein from the chromatography material,

or

iii)

a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,

b) applying the mixture of step a) to a chromatography material,

c) applying a solution which comprises a polysorbate to the chromatography material, and

d) recovering the protein from the chromatography material.

In a further example of all aspects the impurity is a hydrolase.

In a further example of all aspects the impurity is an esterase.

In a further example of all aspects the polysorbate is selected from the group comprising polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65 or polysorbate 80.

In a further example of all aspects the polysorbate is polysorbate 20 or polysorbate 80.

In a further example of all aspects the polysorbate is polysorbate 20.

In a further example of all aspects the polysorbate is polysorbate 80.

In a further example of all aspects the polysorbate is added to a final concentration of at least about 0.001% (w/v).

In a further example of all aspects the polysorbate is added to a final concentration of from about 0.001% (w/v) to about 10% (w/v).

In a further example of all aspects the chromatography material is an affinity chromatography material, a cation exchange chromatography material, an anion exchange chromatography material, a hydrophobic interaction chromatography material or a mixed mode chromatography material.

In a further example of all aspects the chromatography material is an affinity chromatography material or a cation exchange chromatography material.

In a further example of all aspects the chromatography material is an affinity chromatography material or a strong cation exchange chromatography material.

In a further example of all aspects the chromatography material is an affinity chromatography material selected from Protein A chromatography or a chromatography material with a camelid-derived single domain antibody fragment as ligand.

In a further example of all aspects the affinity chromatography material is CaptureSelect FcXL or MabSelect SuRe.

In a further example of all aspects the cation exchange chromatography material is SP Sepharose Fast Flow or Poros XS or Poros 50 HS.

In a further example of all aspects the protein is a monoclonal antibody.

In a further example of all aspects the protein is a humanized Type II anti-CD20 antibody or an anti-VEGF/Ang2 antibody or an anti-HER2 antibody.

One aspect as reported herein is a liquid antibody preparation with reduced hydrolytic activity (increased polysorbate degradation stability/reduced polysorbate degradation) obtained by a method as reported herein.

In an example, there is provided a liquid antibody preparation with increased stability obtained by a method as reported herein.

In an example, there is provided a liquid antibody preparation with reduced particle formation (by free fatty acid assemblies) obtained by a method as reported herein.

One aspect as reported herein is a method for producing a protein comprising the following steps:

a) cultivating a mammalian cell comprising a nucleic acid encoding the protein,

b) recovering the protein from the cell or the cultivation medium, and

c) purifying the protein with a method as reported herein,

and thereby producing the protein.

One aspect as reported herein is a liquid composition comprising an antibody and a polysorbate, wherein the polysorbate is degraded during storage of the liquid composition by 20% or less per year.

In one example the antibody is a humanized Type II anti-CD20 antibody, or an anti-VEGF/Ang2 antibody or an anti-HER2 antibody. In one embodiment the antibody is obinutuzumab, faricimab, trastuzumab or pertuzumab.

Detailed Description of the Invention

Herein is reported a method for purifying a protein by contacting a sample comprising the protein (and an impurity with hydrolytic activity) with a polysorbate, like Polysorbate 20 (PS20), before and/or during a chromatographic step.

It has been found that a protein can be purified from a sample by using a polysorbate as an additive in a purification step, such as e.g. a chromatography step. In particular, impurities with hydrolytic activity like hydrolyzing enzymes (e.g. lipases) can be reduced.

Consequently, one aspect as reported herein is a method for purifying a protein from a sample comprising the protein and at least one impurity with hydrolytic activity, comprising the following steps:

i)

a) applying the sample comprising the protein and at least one impurity with hydrolytic activity to a chromatography material (under conditions suitable that the protein is bound by the chromatography material),

b) applying a (washing) solution which comprises a polysorbate/a polysorbate-like non-ionic surfactant to the chromatography material (under conditions suitable that the protein is bound by the chromatography material), and

c) recovering the protein from the chromatography material,

or

ii)

a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,