chromatographic step, to inhibit or reduce particle formation in a pharmaceutical formulation. 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 chromatographic step, to inhibit or reduce polysorbate degradation in a pharmaceutical formulation.

It has been found that by the methods and uses of the invention the hydrolytic activity in a sample can be reduced significantly. A sample comprising the protein to be purified and an impurity with hydrolytic activity is contacted or mixed with a non-ionic surfactant like a polysorbate either before the sample is loaded onto a chromatographic material, or during the sample is purified in a chromatographic step or both, before and during a chromatographic step. In all these situations the hydrolytic activity, e.g. resulting in polysorbate degradation, can be reduced.

When compared to a sample that is purified for example with a chromatographic step without the use of a polysorbate before and/or during the chromatographic step, it was found that the hydrolytic activity (especially with respect to polysorbate hydrolysis) is considerably lowered in a sample that is contacted or mixed with a polysorbate in the purification procedure. Thus, it was found that polysorbate degradation (by hydrolysis) in a purified sample (e.g. an antibody preparation/formulation) can be reduced by implementing a step of using indeed polysorbate as an additive in a preceding purification procedure. Without being bound by this theory, interestingly, the reduction of impurities, like an enzyme with hydrolytic activity, such as an esterase, is therefore performed/achieved by adding the substrate or target of the impurity (that may later, for example during storage, be subject to hydrolytic cleavage) in/during the purification procedure.

Thus, 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 a sample comprising the protein and at least one impurity with hydrolytic activity to a chromatography material,

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

c) recovering the protein from the chromatography material,

or

ii)

a) adding a polysorbate to a 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 a 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 (washing) solution which comprises a polysorbate to the chromatography material, and

d) recovering the protein from the chromatography material.

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) 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

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,

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

d) recovering the protein from the chromatography material.

Reported herein is a method for reducing the hydrolytic activity in a sample comprising a 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,

b) applying a solution which comprises a polysorbate to 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,

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.

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

i)

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

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,

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).

Patent Claims

1. 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) 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

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,

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

d) recovering the protein from the chromatography material.

2. A method for reducing the hydrolytic activity in a sample comprising a protein and at least one impurity with hydrolytic activity, comprising the following steps:

i)

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

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,

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

d) recovering the protein from the chromatography material.

3. The method according to any one of claims 1 to 2, wherein the impurity is a hydrolase.

4. The method according to any one of claims 1 to 3, wherein the impurity is an esterase.

5. The method according to any one of claims 1 to 4, wherein the polysorbate is selected from the group comprising polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65 or polysorbate 80.

6. The method according to any one of claims 1 to 5, wherein the polysorbate is polysorbate 20 or polysorbate 80.

7. The method according to any one of claims 1 to 6, wherein the polysorbate is added to a final concentration of at least 0.001% (w/v).

8. The method according to any one of claims 1 to 7, wherein the polysorbate is added to a final concentration of from 0.001% (w/v) to 10% (w/v).

9. The method according to any one of claims 1 to 8, wherein 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.

10. The method according to any one of claims 1 to 9, wherein the chromatography material is an affinity chromatography material or a cation exchange chromatography material.

11. The method according to any one of claims 1 to 10, wherein 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.

12. The method according to any one of claims 1 to 11, wherein the affinity chromatography material is CaptureSelect FcXL or MabSelect SuRe.

13. The method according to any one of claims 1 to 10, wherein the cation exchange chromatography material is SP Sepharose Fast Flow or Poros 50HS or Poros XS.

14. The method according to any one of claims 1 to 13, wherein the protein is a monoclonal antibody.

15. The method according to any one of claims 1 to 14, wherein the protein is a humanized Type II anti-CD20 antibody, or an anti-VEGF/Ang2 antibody or an anti-HER2 antibody.

16. 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 according to any one of claims 1 to 15,

and thereby producing the protein.