## Summary

Herein is reported a method for the purification or production of a hydrophilic antibody with a mixed mode/multimodal chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups in the presence of an antichaotropic salt.

The present disclosure is based, at least in part, on the unexpected finding that antibody related high molecular weight impurities (HMWs) can be successfully reduced when the load solution comprising a hydrophilic antibody and HMWs is purified by a mixed mode chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups (MM HIC/IEX) in flowthrough (FT) mode in the presence of at least one antichaotropic salt. In one preferred example of the disclosure, the antichaotropic salt is present in the equilibration (buffer) used to equilibrate the chromatography material, the load solution and optional washing/rising solutions. It has been found that the production and/or purification, i.e. the reduction of HMWs, can be performed for hydrophilic antibodies in the presence of an antichaotropic salt but that the effect cannot be achieved for hydrophobic antibodies or in the presence of a chaotropic salt.

Further, the present disclosure is based, at least in part, on the unexpected finding that also contaminations with viruses or virus-like particles (e.g. RVLPs) i.e. the viral impurity content can be successfully reduced when the load solution comprising a hydrophilic antibody and a viral impurity is purified by a mixed mode chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups (MM HIC/IEX) in flowthrough (FT) mode in the presence of at least one antichaotropic salt. In one preferred example of the disclosure, the antichaotropic salt is present in the equilibration (buffer) used to equilibrate the chromatography material, the load solution and optional washing/rising solutions. It has been found that the production and/or purification, i.e. the reduction of viral impurity content, can be performed for hydrophilic antibodies in the presence of an antichaotropic salt but that the effect is not present for hydrophobic antibodies.

Thus, the present disclosure provides a method for producing an antibody using (/with) a mixed mode/multimodal chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups (MM HIC/IEX) operated in flowthrough mode, wherein

a) the antibody is a hydrophilic antibody, and

b) the antibody is applied in a solution comprising the antibody and an antichaotropic salt to the MM HIC/IEX.

In one aspect, the present disclosure provides a method for producing an antibody using (with) a mixed mode chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups (MM HIC/IEX) operated in flowthrough mode, wherein

a) the antibody is a hydrophilic antibody, and

b) the antibody is applied in a solution comprising the antibody and an antichaotropic salt to the MM HIC/IEX.

In certain examples, the method further comprises the following steps:

c) optionally a rinsing solution is applied,

d) the antibody is recovered in the flowthrough of b) or optionally in the flowthrough of b) and c),

and thereby producing the antibody using a MM HIC/IEX operated in flowthrough mode.

The present disclosure provides a method for purifying an antibody using (with) a mixed mode/multimodal chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups (MM HIC/IEX) operated in flowthrough mode, wherein

a) the antibody is a hydrophilic antibody, and

b) the antibody is applied in a solution comprising the antibody and an antichaotropic salt to the MM HIC/IEX,

and thereby purifying the antibody.

In certain examples, the MM HIC/IEX has been conditioned/equilibrated with a buffer comprising the (same) antichaotropic salt. In one preferred example, the buffer used for conditioning/equilibrating the MM HIC/IEX is also the buffer of the solution of step b).

In certain examples,

- the method is for producing an antibody composition with reduced antibody-related high molecular weight (HMW) impurity content and/or with reduced viral impurity content,

- the antibody is applied to the MM HIC/IEX in a solution comprising the antibody, at least one antibody-related HMW impurity and/or at least one viral impurity and an antichaotropic salt,

- the antibody composition with reduced antibody-related HMW impurity content and/or with reduced viral impurity content is recovered from the flowthrough, and

- thereby an antibody composition with reduced antibody-related HMW impurity content and/or with reduced viral impurity content is produced.

In certain examples,

- the method is for producing an antibody composition with reduced antibody-related high molecular weight (HMW) impurity content and with reduced viral impurity content,

- the antibody is applied to the MM HIC/IEX in a solution comprising the antibody, at least one antibody-related HMW impurity and at least one viral impurity and an antichaotropic salt,

- the antibody composition with reduced antibody-related HMW impurity content and with reduced viral impurity content is recovered from the flowthrough, and

- thereby an antibody composition with reduced antibody-related HMW impurity content and with reduced viral impurity content is produced.

In certain examples,

- the method is for producing an antibody composition with reduced antibody-related high molecular weight (HMW) impurity content,

- the antibody is applied to the MM HIC/IEX in a solution comprising the antibody, at least one antibody-related HMW impurity and an antichaotropic salt,

- the antibody composition with reduced antibody-related HMW impurity content is recovered from the flowthrough, and

- thereby an antibody composition with reduced antibody-related HMW impurity content is produced.

In certain examples, the antibody-related (HMW) impurity content is reduced compared to the solution applied to the MM HIC/IEX in step b).

In certain examples, the antibody-related (HMW) impurity content is reduced compared to a solution essentially without an antichaotropic salt; and/or compared to a solution comprising a hydrophobic antibody.

In certain examples,

- the method is for producing an antibody composition with reduced viral impurity content,
- the antibody is applied to the MM HIC/IEX in a solution comprising the antibody, at least one viral impurity and an antichaotropic salt,
- the antibody composition with reduced viral impurity content is recovered from the flowthrough, and
- thereby an antibody composition with reduced viral impurity content is produced.

In certain examples, the viral impurity content is reduced compared to the solution applied to the MM HIC/IEX in step b).

In certain examples, the viral impurity content is reduced compared to a solution essentially without an antichaotropic salt; and/or compared to a solution comprising a hydrophobic antibody.

In certain examples, the hydrophilic antibody is an antibody that has a retention time on a hydrophobic interaction chromatography (HIC) material that is equal or less than that of rituximab (on the same HIC material and under the same operating conditions).

In certain examples, the hydrophobic interaction chromatography (HIC) material contains polyether groups (ethyl ether groups) as ligand.

In certain examples, the hydrophobic interaction chromatography (HIC) material contains polyether groups with the following structure (-$(OCH_2CH_2)_nOH$) as ligand.

In certain examples, the hydrophobic interaction chromatography (HIC) material contains a polymethacrylate base material/matrix.

In certain examples, the hydrophobic interaction chromatography (HIC) material contains polyether groups (ethyl ether groups) as ligand, has a mean pore size of 100 nm and a particle size of 10 µm.

In certain examples, the hydrophobic interaction chromatography (HIC) material is a TSKgel® Ether-5PW chromatography material.

In certain examples, the retention time on the HIC chromatography material is determined using the HIC chromatography material (of any one of the other examples), and with a column length of 75 mm, and with an inner diameter of 7.5 mm, and with an elution buffer gradient at a flow rate of 8.8 ml/min, and wherein the antibody is applied to the chromatography material at a concentration of 1 mg/ml.

In certain examples, the antichaotropic salt has a molar surface tension increment in the range of and including 1.285 to 4.183 x 10E3 $dyn*g*cm^{-1}*mol^{-1}$.

In certain examples, the antichaotropic salt is selected from the group consisting of chlorides, sulfates, citrates, carbonates, phosphates, acetates or fluorides.

In certain examples, the antichaotropic salt is a calcium-, sodium-, ammonium- or potassium-salt.

In certain examples, the antichaotropic salt is a sodium-, ammonium- or potassium-salt.

In certain examples, the antichaotropic salt is selected from the group consisting of $(NH_4)_2SO_4$, $Na_2SO_4$, $K_2SO_4$, NaCl and KCl.

In certain examples, the solution comprising the antibody and an antichaotropic salt (of step b) has a conductivity of from (and including) 0.5 to 120 mS/cm.

In certain examples, in the solution comprising the antibody and an antichaotropic salt, the antichaotropic salt has a concentration of from (and including) 10 mM to 900 mM.

In certain examples, the loaded amount to the MM HIC/IEX is 10 g of protein per Liter of chromatography material (10 g/L) or higher.

In certain examples, the loaded amount to the MM HIC/IEX is from (and including) 10 g of protein per Liter of chromatography material (10 g/L) to 650 g of protein per Liter of chromatography material (650 g/L).

In certain examples, the loaded amount to the MM HIC/IEX is from (and including) 15 g of protein per Liter of chromatography material (15 g/L) to 350 g of protein per Liter of chromatography material (350 g/L).

In certain examples, the solution comprising the antibody and an antichaotropic salt has a pH value of from (and including) 4.0 to 9.0.

In certain examples, the HMW impurity is an impurity which has a molecular weight of 285 kDa or more.

In certain examples, the HMW impurity is an impurity which is at least a dimer, or a trimer, or any multimer of the antibody.

In certain examples, the MM HIC/IEX comprises anion exchange functional groups or cation exchange functional groups.

In certain examples, the MM HIC/IEX comprises strong anion exchange functional groups.

In certain examples, the MM HIC/IEX is Capto™ adhere ImpRes, Capto™ Adhere or Nuvia aPrime4A.

In certain examples, the MM HIC/IEX comprises weak cation exchange functional groups.

In certain examples, the MM HIC/IEX is Capto™ MMC or Capto™ MMC ImpRes.

## Detailed Description of the Invention

Herein is reported a method for the purification or production of a hydrophilic antibody using a mixed mode/multimodal chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups and with the use of an antichaotropic salt in the load solution (denoted also as "load" herein).

The present invention is based, at least in part, on the unexpected finding that antibody related high molecular weight impurities (HMWs) can be successfully reduced when the load comprising a hydrophilic antibody and HMWs is purified by a mixed mode chromatography material that comprises ion exchange functional groups and hydrophobic interaction functional groups (MM HIC/IEX) in flowthrough (FT) mode and there is at least one antichaotropic salt comprised in the load solution prior to the start of the chromatography.

In more detail and surprisingly, it has been found that the content of HMWs can be reduced significantly more, when the to-be-purified antibody is a hydrophilic antibody and an antichaotropic salt is present. In contrast, when hydrophobic antibodies are being purified, no significant effect of the addition of an antichaotropic salt regarding HMW reduction can be observed.