Solvent modulation of column chromatography is reported by Arakawa, T., et al. in Prot. Pep. Lett. 15 (2008) 544-555. In EP 1 729 867 a method for chromatographic purification is reported. Antibody aggregate removal by hydroxyapatite chromatography is reported by Gagnon, P. and Beam, K., in Curr. Pharm. Biotechnol. 10 (2009) 440-446. In J. Immunol. Meth. (336 (2008) 222-228) reports Gagnon, P., an improved antibody aggregate removal by hydroxyapatite chromatography in the presence of polyethylene glycol. In WO 2004/013162 an increased dynamic binding capacity in ion exchange chromatography by addition of polyethylene glycol is reported. In WO 2005/094960 a method for chromatographic purification is reported. Enhanced capacity and purification of antibodies by mixed mode chromatography in the presence of aqueous-soluble non-ionic organic polymers is reported in WO 2008/086335. In WO 2009/149067 a process for the purification of antibodies is reported.

Milby, K.H., et al. report the ion-exchange chromatography of proteins and the effect of neutral polymers in the mobile phase (J. Chrom. 482 (1989) 133-144). In Biotechnology Techniques (12 (1998) 289-293) Feng, X-L., et al. report that polyethylene glycol improves the purification of recombinant human tumor necrosis factor during ion exchange chromatography. A method for obtaining unique selectivities in ion-exchange chromatography by addition of organic polymers to the mobile phase is reported by Gagnon, P., et al. (J. Chrom. 743 (1996) 51-55). Gagnon, P., reports in an internet article on validated.com (http://www.validated.com/revalbio/pdffiles/ionslect.pdf) the fine-tuning of selectivity on ion exchangers. In WO 2009/149067 a process for the purification of antibodies is reported.

Summary ~~of the Invention~~

It has been found that high PEG-concentrations can be employed during cation exchange chromatography in the presence of certain additives (solubility enhancers) in order to modulate selectivity and to improve resolution of antibody monomer and aggregate species, e.g. compared to methods employing PEG-only.

Herein is reported a method for isolating or obtaining or producing a polypeptide of interest in monomeric form from other component(s), such as the polypeptide of interest in aggregated form, comprising at least one chromatographic step. In one embodiment the method comprises contacting a solution comprising the polypeptide of interest with a cation-exchange chromatography material wherein

the contacting with the chromatography material takes place in the absence of a non-ionic polymer and an additive, and wherein the recovering takes place in the presence of a non-ionic polymer and an additive.

It has been found that by using an additional additive in a method as reported herein the effect of the non-ionic polymer can be improved.

As~~One aspect as~~ reported herein is a method for producing an antibody in monomeric form comprising the following steps:

- applying a solution comprising a non-ionic polymer and an additive to a chromatography material to which an antibody had been adsorbed, whereby the antibody in monomeric form remains adsorbed to the ion exchange chromatography material, and
- recovering the antibody in monomeric form from the ion exchange chromatography material by applying a solution comprising a non-ionic polymer, an additive, and an elution compound, and thereby producing the antibody in monomeric form.

In a first aspect, there is provided a method for producing an antibody of the IgG class in monomeric form comprising the following steps:

- applying a first solution that optionally comprises poly (ethylene glycol) and sorbitol to a cation exchange chromatographic material and thereby equilibrating the material,

- applying the solution comprising the antibody of the IgG class to the equilibrated chromatography material and thereby loading the chromatography material,

- producing the antibody of the IgG class in monomeric form by applying a solution to the chromatographic material comprising poly (ethylene glycol) and sorbitol and thereby desorbing/eluting the antibody of the IgG class in monomeric form from the chromatographic material,

whereby the poly (ethylene glycol) has a concentration of 10 % by weight and the sorbitol has a concentration of from 5 % to 20 % by weight.

AsAnother aspect as reported herein is a method for producing a polypeptide preparation with reduced host cell protein content whereby the preparation is obtained from a mammalian cell cultivation supernatant, especially a CHO cell cultivation supernatant, comprising the following steps:

- applying a solution comprising a non-ionic polymer and an additive to a chromatography material to which the polypeptide had been adsorbed, whereby the polypeptide in monomeric form remains adsorbed to the ion exchange chromatography material, and
- recovering the polypeptide in monomeric form from the ion exchange chromatography material by applying a solution comprising a non-ionic polymer, an additive, and an elution compound, and thereby producing the polypeptide preparation with reduced host cell protein content.

In a second aspect, there is provided a method for producing an antibody of the IgG class preparation with reduced host cell protein content comprising the following steps:

- applying a first solution that optionally comprises poly (ethylene glycol) and sorbitol to a cation exchange chromatographic material and thereby equilibrating the material,

- applying the solution comprising the antibody of the IgG class to the equilibrated chromatography material and thereby loading the chromatography material,

- producing the antibody of the IgG class in monomeric form by applying a solution to the chromatographic material comprising poly (ethylene glycol) and sorbitol and thereby desorbing/eluting the antibody of the IgG class in monomeric form from the chromatographic material,

whereby the poly (ethylene glycol) has a concentration of 10 % by weight and the sorbitol has a concentration of from 5 % to 20 % by weight.

AsOne aspect as reported herein is a method for determining the concentration of a non-ionic polymer and an additive for use in an ion exchange chromatography of a polypeptide comprising the following steps:

- determining in solution in the absence of the additive the concentration of the non-ionic polymer at which less than 50 % of the polypeptide remain in solution,
- determining in solution in the presence of a concentration of the non-ionic polymer, which has been determined in the previous step, the concentration of the additive at which more than 95 % of the polypeptide remain in solution,

thereby determining the concentration of a non-ionic polymer and an additive.

As~~One aspect as~~ reported herein is a method for producing an antibody in monomeric form comprising the following step:

- recovering the antibody in monomeric form from an ion exchange chromatography material by applying a solution comprising a non-ionic polymer, an additive, and an elution compound, and thereby producing the antibody in monomeric form,

wherein the separation efficiency (resolution) of the chromatography is enhanced compared to a chromatography in the absence of the non-ionic polymer, and

wherein the employed concentration of the non-ionic polymer would result in a partial or complete precipitation of the antibody in the absence of the additive.

In one embodiment the ion exchange chromatography material is a cation exchange chromatography material.

In one embodiment the non-ionic polymer is selected from the group comprising poly (ethylene glycol) (PEG), poly (propylene glycol) (PPG), PEG-PPG copolymers, and triblock copolymers composed of poly (oxypropylene) (poly (propylene oxide)) flanked by poly (oxyethylene) (poly (ethylene oxide)). In a further embodiment the non-ionic polymer is poly (ethylene glycol).

In one embodiment the additive is selected from the group comprising zwitterions, amino acids, urea, urea derivatives, ampholytes, CHAPSO, natural products, sugars, and polyols. In a further embodiment the additive is sorbitol.