and a C-terminal Fc fragment, in particular a murine IgG2a or human IgG1 (see, e.g., WO 97/41232; WO 2004/035622; Kim, Y.S., et al., J. Immunol. 160 (1998) 5742-5748).

In general the IEP-values (isoelectric point, pI) of proteins are important characteristics for ion exchange chromatography of proteins. Below the IEP proteins gain a positive net charge and can be chromatographed on cation exchangers, above the pI they have a negative net charge and anion exchangers should be used (see e.g. New comprehensive biochemistry, Neuberger, A., and van Deenen, L.L.M. (eds.), Volume 8, Separation Methods, Deyl, Z. (ed.), Elsevier Science Publishers B.V. (1984) page 247).

Conjugated Factor VIII molecules are reported in WO 2009/108806. In US 2009/0304669 a preparative purification process for human furin is reported. A chromatography purification of antibodies is reported in US 2009/270596. In WO 2009/074634 is reported a composition for pulmonary delivery. Polypeptides, antibody variable domains and antagonists are reported in WO 2008/149147. In US 2008/177048 an enhanced capacity and purification of antibodies by mixed-mode chromatography in the presence of aqueous-soluble non-ionic organic polymers is reported. Refolding of recombinant proteins is reported in US 2008/008975. In US 2007/167613 a process for purification of antibodies is reported. Antibody purification is reported in US 2007/112178. Purified immunoglobulin fusion proteins and methods of their purification are reported in WO 2009/111347.

Aldington et al. (J. Chrom. B 848 (2007) 64-78) report the scale-up of monoclonal antibody purification processes. Pilot scale purification of human monoclonal IgM (COU-1) is reported by Tornoe at al. (J. Immunol. Meth. 205 (1997) 11-17). In US 2010/069617 enhanced protein aggregate removal by mixed-mode chromatography on hydrophobic interaction media in the presence of protein-excluded zwitterions is reported.

Summary

Herein is reported a method for purifying a polypeptide or immunoglobulin wherein the starting solution comprises an aggregate content of 20 % or more as determined by size exclusion chromatography, i.e. for removing aggregates from a crude polypeptide or immunoglobulin solution with high aggregate content.

The method is especially useful for solutions containing a high aggregate burden of at least 20 % as determined by size-exclusion chromatography.

The high aggregate burden can be reduced to low levels with a combination of two chromatography steps. It has been found that a combination of two chromatography steps provides for improved aggregate removal and at the same time for an increased recovery compared to a single chromatography step for the purification of crude polypeptide or immunoglobulin solution comprising more than 20 % of the polypeptide or immunoglobulin in aggregated form.

It has been found that a mixed-mode chromatography material and a weak cation exchange chromatography material can be operated at pH values above, i.e. higher than, the isoelectric point (IEP) of the polypeptide of interest in order to obtain the polypeptide in monomeric form, i.e. in order to separate it from the polypeptide in aggregated form.

As reported herein is a method for producing a polypeptide in monomeric form, i.e. a monomeric polypeptide, comprising the steps

a) i) applying a solution comprising the polypeptide in monomeric form and in aggregated form, wherein the aggregated form is 20 % or more as determined by size exclusion chromatography of the total polypeptide in the solution, to a hydrophobic interaction chromatography material and recovering the polypeptide from the flow-through, or

ii) applying a solution comprising the polypeptide in monomeric form and in aggregated form, wherein the aggregated form is 20 % or more as determined by size exclusion chromatography of the total polypeptide in the solution, to a mixed-mode chromatography material and recovering the polypeptide from the mixed-mode chromatography material,

and

b) applying the solution obtained in the previous step to a weak cation exchange chromatography material and recovering the polypeptide therefrom and thereby producing the polypeptide in monomeric form, i.e. the monomeric polypeptide.

One aspect as reported herein is a method for producing a monomeric Fc-region containing polypeptide comprising the steps

a) applying a solution comprising the Fc-region containing polypeptide in monomeric form and in aggregated form, wherein the aggregated form is 20 % or more as determined by size exclusion chromatography of the total Fc-region containing polypeptide in the solution, to a mixed-mode chromatography material and recovering the Fc-region containing polypeptide from the mixed-mode chromatography material,

and

b) applying the solution obtained in the previous step to a weak cation exchange chromatography material and recovering the Fc-region containing polypeptide from the flow-through of the weak cation exchange chromatography material and thereby producing the monomeric Fc-region containing polypeptide.

In one embodiment the pH value of the solution prior to the application to the mixed-mode chromatography material or the weak cation exchange