precipitation step, or where salt has been added to a sample for any reason.

[0004] Methods have been described for processing IgM-containing cell culture harvests that particularly remove chromatin catabolites (Gan *et al* J. Chromatogr. A, 1291 (2013) 33-40). These methods particularly describe the use of the DNA intercalating compound ethacridine for clarifying an IgG-containing cell culture harvest, under roughly physiological conditions.

[0005] Partial purification of monoclonal IgG antibodies by contaminant co-precipitation with caprylic acid (octanoic acid) has been disclosed (Chantuin, A., *et al*, Arch. Biochem. Biophys. 89 (1960) 218-220). The fatty acid binds to all proteins but selectively precipitates non-IgG contaminants (Gagnon, P., Purification Tools for Monoclonal Antibodies, 1996, Validated Biosystems, Tucson; Morais, V., *et al*, Biotechnol. Appl. Biochem., 59 (2012) 50-54). Process development guidelines for application to cell-free cell culture harvests have been indicated (Gagnon *supra*). Application of caprylic acid to cell-containing cell culture harvests has been described (Brodsky *et al* Biotechnol. Bioeng. 109 (2012) 2589-2598). The technique has the unfortunate feature of co-producing a turbid, sticky, electronegative haze that interferes with further purification (Gagnon *supra*; Brodsky *et al supra*).

[0006] Allantoin is an FDA-approved inflammatory agent used widely in over-the-counter healthcare products. It is known to remove endotoxin from protein solutions, including from solutions of IgG (V. Vagenende et al, ACS. Appl. Mater. Interfaces, 22 (2013) 4472-4478; V. Vagenende et al, J. Chromatogr. A 1310 (2013) 15-20).

SUMMARY

[0007] In one aspect, the present invention refers to a method of purifying an IgG antibody from a preparation containing the antibody and contaminants comprising: providing the preparation in a form having less than 5% of the chromatin residing in a source sample from which the preparation was obtained, and contacting the preparation with an electropositive membrane having a porosity that retains at least 50% of non-adsorbed solutes with a hydrodynamic diameter greater than a selected size but permits passage of non-adsorbed solutes with a hydrodynamic diameter less than the selected size, wherein the selected size is selected from between about 10 nm and about 15 nm, further wherein during at least a portion of the contacting step the preparation comprises a salt such that (1) when the salt is present at a concentration less than about 50 mM, a pH value of the preparation is in a range from about 3 to

within about 0.5 pH units of the isoelectric point of the most alkaline glycoform of the IgG antibody in the preparation; or (2) when the salt is present at concentration greater than about 50 mM, a pH value of the preparation is in a range from about 3 to about 9; and wherein the final operating condition of the contacting step is defined by either an absence of excess salt in the preparation or a non-zero salt concentration in the preparation of not greater than 20 mM, and a pH value in a range from about 5 to within about 0.5 pH units of the isoelectric point of the most alkaline glycoform of the IgG antibody. In another aspect, the present invention refers to a kit for practicing the method of the present invention. ~~In certain aspects, the invention provides~~Also disclosed herein are methods of purifying an IgG antibody from a preparation containing the antibody and contaminants, preferably where the preparation has been processed to remove at least 95% of the chromatin present in the original production medium from which it is derived. The method includes a step of contacting the preparation with an electropositive membrane having a porosity that retains at least 50% of non-adsorbed solutes with a hydrodynamic diameter greater than a selected size but permits passage of non-adsorbed solutes with a hydrodynamic diameter less than the selected size and the selected size may be any amount between about 10 nm and about 15 nm. During at least a portion of the contacting step the preparation comprises a salt such that (1) when the salt is present at a concentration less than about 50 mM, a pH value of the preparation is in a range from about 3 to within about 0.5 pH units of the isoelectric point of the most alkaline glycoform of the IgG antibody in the preparation; or (2) when the salt is present at concentration greater than about 50 mM, a pH value of the preparation is in a range from about 3 to about 9. The final operating condition of the contacting step is defined by either an absence of excess salt in the preparation or a non-zero salt concentration in the preparation of not greater than 20 mM, and a pH value in a range from about 5 to within about 0.5 pH units of the isoelectric point of the most alkaline glycoform of the IgG antibody.

[0008] In certain ~~embodiments~~examples, the preparation is provided for the method in a form having a reduced level of chromatin such that the amount of chromatin in the preparation is less than about 5% of the amount of chromatin which had been present in the source sample from which the preparation was obtained. In certain ~~aspects~~examples, the source sample is a cell culture harvest, tissue sample, or bodily fluid having a substantial amount of chromatin present and the source sample is subjected to one or more processes for clarification, purification or

fractionation to obtain the preparation having less than about 5% of the chromatin of the source sample for use in a method ~~of the invention~~as described herein.

DETAILED DESCRIPTION

[0009] With respect to certain aspects of the invention it has been discovered that surprising improvements in the level of purification of preparations containing IgG antibodies is achievable with the performance of a chromatin-directed clarification followed by a fractionation process employing charged membranes having a porosity sufficient to retain the antibodies. Thus in certain aspects the purification methods of the invention are directed to preparations containing IgG antibodies where the preparation has been obtained through a process or processes which resulted in the substantial reduction in the levels of chromatin in comparison with its original source. For example, in certain embodiments the original source sample providing the desired IgG antibody is cell culture harvest, bodily fluid or tissue sample and the original source sample is subjected to steps for substantial reduction of chromatin levels; in certain such embodiments the chromatin levels of the original source sample are reduced by over 95% such that the preparation provided for the methods of the invention has less than 5% of the chromatin originally present in the source sample. Levels of chromatin reduction may be assessed with reference to the levels in the original source sample and in the preparation provided in the