PURIFICATION OF BIOLOGICAL PRODUCTS BY CONSTRAINED COHYDRATION CHROMATOGRAPHY

[0100] This application claims priority to U.S. provisional application 61/494,669, filed on June 8, 2011, U.S. provisional application 61/494,687, filed on June 8, 2011, Singapore Patent Application No. 201108397-9, filed on November 14, 2011 and U.S. provisional application 61/653,950, filed on May 31, 2012~~, each of which are incorporated herein by reference in their entireties~~.

FIELD OF THE INVENTION

[0101] This invention relates to methods for enhancing purification of biological products. It relates particularly to methods for enhancing purification of virus and purification of antibodies.

BACKGROUND OF THE INVENTION

[0102] Chromatography methods typically rely on exploitation of a solid surface that bears at least one chemical functionality to actively engage in interactions with biomolecules in order to sort the components of a complex sample according to that chemical functionality. These methods are called adsorptive chromatography methods. Surface chemistries differ among adsorptive methods, along with the chemical means to release bound components, but the operational format is the same. One example is bioaffinity chromatography, in which an inert surface is substituted with a biological ligand specific for a component of interest from a complex sample. The component of interest binds, the rest do not, and the component of interest is subsequently released by changing the chemical conditions. Ion exchange chromatography is representative of a greater diversity of methods. An inert surface is substituted covalently with a charged chemical group. In the case of anion exchange chromatography, the chemical group is positively charged. Sample components of sufficient negative charge bind, with the most negatively charged binding most strongly. Sample components lacking sufficient negative charge fail to bind. Bound components can be released in order of the strength of their interaction by the application of an increasing gradient of salt, which imposes a gradually increasing degree of disruption to the charge interactions, releasing the bound components in order of the intensity of their interaction with the anon exchanger. Except for differences of chemistry, the same operational format is applied to cation exchange chromatography, hydrophobic interaction chromatography, reversed-phase chromatography, and numerous examples of so-called mixed-mode chromatography, including hydroxyapatite.

fulfilled this requirement to date. All others require substantial modification of conditions and/or are rendered ineffective by components of cell culture supernatants.

[0109] References:

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(3) Arakawa, T. and Timasheff, S. N. (1983). Preferential interactions of proteins with solvent components in aqueous amino acid solutions. *Arch. Biochem. Biophys.* 224, 169-177.

(4) Arakawa, T. and Timasheff, S. N. (1985). Mechanism of poly(ethylene glycol) interaction with proteins. *Biochemistry* 24, 6756-6762.

(5) Bhat, R., Timasheff, S., Steric exclusion is the principal source of preferential hydration of proteins in the presence of polyethylene glycols. *Prot. Sci.*, **1**, 113-1143 (1992).

(6) P. Gagnon, (1996) Purification Tools for Monoclonal Antibodies, Validated Biosystems, Tucson.

(7) von der Haar, F., (1976) Purification of proteins by fractional interfacial salting out on unsubstituted agarose gels. *Biochem. Biophys. Res. Comm.*, 70 (3) 1009-1013.

(8) P. Gagnon, B. Godfrey, D. Ladd, (1996) Method for obtaining unique selectivities in ion-exchange chromatography by addition of organic polymers to the mobile phase, *J. Chromatogr. A* 743 51-55

(9) P. Gagnon (2008) Improved antibody aggregate removal by hydroxyapatite chromatography in the presence of polyethylene glycol, *J. Immunol. Met.* 336 222-228

(10) T. Arakawa (1985) The mechanism of increased elution volume of proteins by polyethylene glycol, *Analyt. Biochem.*, 144 267-268.

SUMMARY OF THE INVENTION

[0110] Methods and compositions and kits adapted for performing the invention are provided. In one aspect, there is provided a method for purification of a hydrated target species of biological origin in a sample comprising the steps of contacting the sample with a hydrated surface of an undissolved material and contacting the sample with a constraining

agent in an amount sufficient to cause at least a fraction of the target species to be retained at the hydrated surface of the undissolved material under conditions providing an absence of a significant chemical attraction between the target species and the surface of the undissolved material, wherein the constraining agent is an aqueous soluble uncharged linear or branched non-ionic polymer wherein the undissolved material is a convective chromatography material comprising a monolith, and wherein the target species is a protein, antibody, clotting factor, cellular organelle, virus, virus-like particle, gene therapy vector or cell. In another aspect, there is provided a kit comprising organic nucleation centers, constraining agents configured for the convenient practice of any of the methods of the present invention. In certain ~~embodiments~~examples, the ~~invention~~disclosure provides a method for purification of a target species of biological origin in a sample including the steps of (i) contacting the sample with the hydrated surface of an undissolved material and (ii) contacting the sample and

undissolved material with a preferentially excluded agent in an amount sufficient to cause at least a fraction of the now preferentially hydrated target species to be retained at the now preferentially hydrated surface of the undissolved material. This form of association is herein also referred to as constrained cohydration; the preferentially excluded agent is sometimes referred to as the constraining agent; and for brevity, preferentially hydrated molecules and surfaces are referred to simply as "hydrated." In certain such ~~embodiments~~examples, more than 50% of the hydrated target species is retained at the hydrated surface of the undissolved material by the constraining agent and in some ~~embodiments~~examples substantially all of the target species is retained. In certain such ~~embodiments~~examples, at least one individual of the hydrated target species is retained at the hydrated surface exclusively by constrained co-hydration caused by the presence of the constraining agent. In certain such ~~embodiments~~examples under such conditions substantially all of the target species is retained at the hydrated surface exclusively by constrained co-hydration caused by the presence of the constraining agent. In certain such ~~embodiments~~examples, the exclusive retention of the hydrated target species at the hydrated surface by constrained co-hydration due to the presence of the constraining agent can be demonstrated by termination of such retention through the removal or reduction of the concentration of the constraining agent.

[0111] In certain ~~embodiments~~examples of the ~~invention~~disclosure, methods and associated compositions and kits are provided for practice ~~of the invention~~ in the context of a tripartite system separation, precipitation method, fluidized bed process or a convective chromatographic process.

BRIEF DESCRIPTION OF THE DRAWINGS

[0112] **Figure 1** shows the relationship between the percentage of IgM retained by constrained co-hydration at the surface of a hydrated monolith and pH for different percentages of the preferentially excluded agent, PEG-6000 (8%, 9%, 10%, 11%, and 12%) as described in Example 5.

[0113] **Figure 2** shows the relationship between the percentage of IgM retained by constrained co-hydration at the surface of a hydrated monolith and concentration of NaCl for different percentages of the preferentially excluded agent, PEG-6000 (8%, 9%, 10%) as described in Example 6.

[0114] **Figure 3** shows the relationship between the percentage of IgM retained by constrained co-hydration at the surface of a hydrated monolith and percentages of a preferentially excluded agent, PEG of varying molecular weights (6000, 4000, and 2000)as described in Example 7.

equivalent to unpurified virus, highlighting the benign influence of the separation method and conditions. (See Figure 6).

[0254] **Example 33**. Monolith-based CCC purification of IgM. OH monoliths were also used to evaluate the ability of constrained cohydration to achieve selective retention of IgM. This class of antibodies is notoriously labile, with a molecular weight of about 1 MDa25. Monoclonal IgM grown in 20% serum- supplemented cell culture media was loaded by in-line dilution at 1 part supernatant to 4 parts 12.5% PEG-6000, yielding a final PEG concentration of 10%. The monolith was washed with 10% PEG, 200 mM NaCl, 50 mM MES, pH 6.0, then eluted with a linear gradient to 50 mM MES, 200 mM NaCl, pH 6.0. CCC was able to achieve 90% purity and 70% recovery of monoclonal IgM, despite the initial antibody concentration being only about 20 µg/mL (Fig. 7). We have since achieved similar CCC results with more than 20 IgM clones grown under the same conditions. Purity increases up to 95% and recovery up to 90% with higher-producing clones grown in protein-free cell culture media. Dynamic binding capacities for different clones range from 20 to 38 mg IgM/mL monolith. Practical capacity is sometimes determined more by pressure than surface availability, since pressure increases linearly throughout the sample loading process. This is believed to result from gradual narrowing of the monolith's channels by accretion of protein on their surfaces or, as shown in figure 6, mediated by virus. (See Figure 7).

[0255] The present invention may be combined with other purification methods to achieve higher levels of purification. Examples include, but are not limited to, other methods commonly used for purification of proteins or virus, such as other precipitation methods, affinity chromatography, anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography, immobilized metal affinity chromatography, and additional mixed mode chromatography methods. It is within the purview of a person of ordinary skill in the art to develop appropriate conditions for the various methods and integrate them with the invention herein to achieve the necessary purification of a particular antibody.

~~**[0256]** All references cited herein are incorporated by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.~~

~~[0257]~~[0256] All numbers expressing quantities of ingredients, operating conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired performance sought to be obtained by the present invention.

~~[0258]~~[0257] Many modifications and variations of this invention can be made without departing from its ~~spirit and~~ scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only and are not meant to be limiting in any way. It is intended that the specification and examples be considered as exemplary only, with the true scope ~~and spirit~~ of the invention being indicated by the following claims.