[0005] In addition to viral removal, membrane filters may be used to remove protein aggregates from biologic drugs. For example, aqueous solutions of antibodies may contain aggregates of antibodies that should be removed prior to administration to a patient to avoid potential toxic responses. These protein aggregates contribute to membrane filter fouling as well as reducing overall yields of the biologic drug.

[0006] Thus, there is a continuing need for better, more economical methods for filtration of biologic solutions to remove potential viral contaminants and reduce protein aggregates. The invention provided herein addresses these needs and provides additional benefits.

[0007] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0008] The present disclosure~~invention~~ provides methods of reducing fouling of ultrafiltration membranes in processes where virus particles are removed from aqueous solutions of protein by adding a surfactant or non-surfactant, non-ionic agent directly to an aqueous protein feedstream prior to ultrafiltration. The methods provide the advantages of enhancing the mass throughput of the ultrafiltration membrane and increasing the lifespan of the ultrafiltration membrane. In addition, the disclosure~~invention~~ provides methods to reduce or prevent the formation of aggregates in acqueous solutions of protein.

[0009] In one aspect, there is ~~invention~~ provideds a methods of reducing fouling of an ultrafiltration membrane in a process wherein virus particles are removed from an aqueous solution comprising said virus particles and at least one protein, the method comprising the steps of a) adding to said aqueous solution a surfactant or a non-surfactant, non-ionic agent selected from the group consisting of a polyethylene glycol, a cellulose derivative, arginine, and a dextran, and b) filtering said aqueous solution comprising said surfactant or said non-surfactant, non-ionic agent through said ultrafiltration membranes, wherein the presence of said surfactant or said non-surfactant, non-ionic agent in said aqueous solution reduces fouling of said ultrafiltration membrane.

[0010] In another aspect, there is ~~invention~~ provideds a methods of enhancing filtration throughput efficiency of an ultrafiltration membrane in a process wherein virus particles are removed from an aqueous solution comprising said virus particles and at least one protein, the method comprising adding a surfactant or a non-surfactant, non-ionic agent selected from the group consisting of a polyethylene glycol, a cellulose derivative, arginine, and a dextran to said aqueous solution before filtering said aqueous solution through said ultrafiltration membranes, wherein the presence of said surfactant or said non-surfactant, non-ionic agent in said aqueous solution enhances the filtration throughput efficiency of said ultrafiltration membrane as compared to in the absense of said surfactant or non-surfactant, non-ionic agent.

[0011] In one aspect, there is ~~invention~~ provideds a methods to dissociate polypeptide aggregates or reduce the formation of polypeptide aggregates in an ultrafiltration feed stream comprising an aqueous solution comprising at least one protein, the method comprising adding a surfactant or a non-surfactant, non-ionic agent selected from the group consisting of a polyethylene glycol, a cellulose derivative, arginine and a dextran to said aqueous solution. In some embodiments, the method further includes an ultrafiltration step.

[0012] In one aspect, there is ~~invention~~ provideds a methods of reducing fouling of an ultrafiltration membrane in a process wherein virus particles are removed from an aqueous solution comprising said virus particles and at least one protein, the method comprising the steps of a) filtering said aqueous solution through a device selected from the group consisting of one or more layers of adsorptive depth filters and one or more layers of charged or surface modified microporous membranes; b) adding a surfactant or non-surfactant, non-ionic agent selected from the group consisting of a polyethylene glycol, a cellulose derivative, arginine and a dextran to said aqueous solution; and c) filtering said aqueous solution comprising said surfactant or said non-surfactant, non-ionic agent through said ultrafiltration membranes, wherein the presence of said surfactant or said non-surfactant, non-ionic agent in said aqueous solution reduces fouling of said ultrafiltration membrane.

[0013] In another aspect, there is ~~invention~~ provideds a methods of reducing fouling of an ultrafiltration membrane in a process wherein virus particles are removed from an aqueous solution comprising said virus particles and at least one protein, the method comprising the steps of a) adding a surfactant or non-surfactant, non-ionic agent selected from the group consisting of a

polyethylene glycol, a cellulose derivative, arginine and a dextran to said aqueous solution, b) filtering said aqueous solution through a device selected from the group consisting of one or more layers of adsorptive depth filters and one or more layers of charged or surface modified microporous membranes; and c) filtering said aqueous solution comprising said surfactant or said non-surfactant, non-ionic agent through said ultrafiltration membranes, wherein the presence of said surfactant or said non-surfactant, non-ionic agent in said aqueous solution reduces fouling of said ultrafiltration membrane.

[0014] In some embodiments of any of the aspects ~~of the invention~~ outlined above, the surfactant is a non-ionic surfactant. Examples of non-ionic surfactants include, but are not limited to polysorbate 20, Triton® X-100, Triton® X-405, lauromacrogol, and polysorbate 80. In some embodiments of any of the aspects ~~of the invention~~ outlined above, the non-ionic surfactant is polysorbate 20.

[0015] In some embodiments of any of the aspects ~~of the invention~~ outlined above, the surfactant or non-surfactant, non-ionic agent is added to the aqueous solution at a concentration of 1-10,000 PPM. In some embodiments, the surfactant or non-surfactant, non-ionic agent is added to the aqueous solution at a concentration of 10-200 PPM.

[0016] In some embodiments of any of the aspects ~~of the invention~~ outlined above, the ultrafiltration membrane is a parvovirus retentive membrane. In some embodiments, the ultrafiltration membrane has a pore size of less than about 100 nm or less. In some embodiments, the ultrafiltration membrane has a pore size of about 20 nm or less. In some embodiments, the step of filtering the aqueous solution is by normal flow filtration.

[0017] In some embodiments of any of the aspects ~~of the invention~~ outlined above, the protein in the aqueous solution is an antibody. In some embodiments, the antibody is a monoclonal or humanized antibody.

[0018] In some embodiments of any of the aspects ~~of the invention~~ outlined above, addition of the surfactant or said non-surfactant, non-ionic agent to said aqueous solution enhances the filtration throughput efficiency of said ultrafiltration membrane by at least 10%. In some embodiments, the addition of the surfactant or the non-surfactant, non-ionic agent to the aqueous

solution enhances the filtration throughput efficiency of said ultrafiltration membrane by at least 50%.

[0019] In some embodiments of any of the aspects ~~of the invention~~ outlined above, the virus particles are parvovirus particles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 shows the effect of polysorbate 20 on ultrafiltration of an aqueous solution comprising an anti-PDL1 antibody. Polysorbate 20 was added to the aqueous, antibody-containing feed stream at 0 ppm (1), 20 ppm (2), 50 ppm (3), 70 ppm (4), 100 ppm (5) and 1000 ppm (6). The throughput of the ultrafiltration membrane (VF) in g/m^2 is plotted against the transmembrane pressure in pounds per square inch

[0021] Figure 2 shows the effect of polysorbate 20 on ultrafiltration of an aqueous solution comprising an anti-VEGF antibody. Polysorbate 20 was added to the aqueous, antibody-containing feed stream at 0 ppm (1), 20 ppm (2), 100 ppm (3), 1000 ppm (4), and 10,000 ppm (5). The throughput of the ultrafiltration membrane (VF) in g/m^2 is plotted against the transmembrane pressure in pounds per square inch.

[0022] Figure 3 shows the effect of polysorbate 20 on ultrafiltration of an aqueous solution comprising an anti-MUC16 antibody. Polysorbate 20 was added to the aqueous, antibody-containing feed stream at 0 ppm (1), 20 ppm (2), 100 ppm (3) and 1000 ppm (4). The throughput of the ultrafiltration membrane (VF) in g/m^2 is plotted against the transmembrane pressure in pounds per square inch.

[0023] Figure 4 shows the effect of no additive (1), 1000 ppm polysorbate 20 (2) or 1000 ppm Triton® X-100 (3) on ultrafiltration of an aqueous solution comprising an anti-DR5 antibody. The throughput of the ultrafiltration membrane (VF) in g/m^2 is plotted against the transmembrane pressure in pounds per square inch.

[0024] Figure 5 shows the effect of Triton® X-100 on ultrafiltration of an aqueous solution comprising an anti-PDL1 antibody. Triton® X-100 was added to the aqueous, antibody-containing feed stream at 0 ppm (1), 20 ppm (2), 200 ppm (3), 300 ppm (4), and 1000 ppm (5).