VIRUS REDUCTION METHOD

BACKGROUND

[0001] Embodiments disclosed herein relate to methods for reduction of viruses and viral polynucleotides in preparations containing a desired protein to be purified.

[0002] Some virus species are known to be inactivated by means of exposure to extreme conditions such as highly acidic pH. These include most species of lipid-enveloped viruses. Some virus species are known to be resistant to such inactivation. These include many non-lipid enveloped virus species, also referred to as protein coat viruses.

[0003] Anion exchange chromatography is commonly used for the purification of proteins, including removal of contaminating virus species. Anion exchangers used for such applications are frequently quaternary amine ion exchangers. These applications are performed at neutral to slightly alkaline pH because they generally support the strongest virus binding under those conditions (C. Curtis et al, Biotechnol. Bioeng. 84 (2003) 179-185), and such conditions are required to remove contaminating host cell proteins in parallel with removing viruses (P. Gagnon, J. Chromatogr. A 1221 (2012) 57-70. Primary amine-based anion exchangers have also been described for removal of virus and host cell protein at neutral to alkaline operating pH (W. Riordan et al., Biotechnol. Prog. 25 (2009) 1695-1702).

SUMMARY

[0004] In some examples, disclosed herein are methods for reducing virus and viral DNA levels in an acidic preparation of a desired protein, the method comprising contacting the acidic preparation of the desired protein with a surface bearing a primary amine, secondary amine, or both. In one aspect, the present invention refers to a method for removing virus or virus DNA from an acidic preparation comprising a desired protein, the method comprising (i) contacting the acidic preparation of the desired protein with a surface bearing a primary amine, secondary amine, or both, wherein the surface is in the form of a particle, a membrane, a monolith, or a hydrogel-coated skeletal framework, and the contacting is at a pH from 2.0 to 4.0; and (ii) removing the surface from the acidic preparation comprising the desired protein.

DETAILED DESCRIPTION

[0005] Methods are provided for enhanced removal of virus and viral DNA from a preparation containing a desired protein, during or following low pH treatment, by contacting a low pH preparation with a solid surface bearing primary and/or secondary amines. As used herein, "low pH" treatment refers to a sufficiently low pH as part of a virus inactivation treatment, *i.e.*, a sufficiently low pH to inactivate at least one virus. Thus, a top end of the pH range will typically not be greater than about 4.0. Thus, in some

CLAIMS

What is claimed is:

1. A method for removing virus or virus DNA from an acidic preparation comprising a desired protein, the method comprising (i) contacting the acidic preparation of the desired protein with a surface bearing a primary amine, secondary amine, or both, wherein the surface is in the form of a particle, a membrane, a monolith, or a hydrogel-coated skeletal framework, and the contacting is at a pH from 2.0 to 4.0; and (ii) removing the surface from the acidic preparation comprising the desired protein.

2. The method of claim 1, wherein an operating pH during the contacting step is in a range selected from the group consisting of (a) from 2.25 to 3.75, (b) from 2.5 to 3.5, (c) from 2.75 to 3.25, (d) from 3.5 to 3.75, and (e) from 3.25 to 3.5.

3. The method of claim 1, wherein a salt concentration during the contacting step is in a range selected from the group consisting of (a) from 0.0 to 0.5 M, (b) from 0.05 to 0.3 M, and (c) from 0.1 to 0.2 M.

4. The method of claim 1, wherein the duration of the contacting step is in a range selected from the group consisting of (a) from 15 to 120 minutes, (b) from 30 to 60 minutes, (c) a non-zero amount less than 30 minutes, (d) a non-zero amount less than 15 minutes, (e) a non-zero amount less than 10 minutes, (f) a non-zero amount less than 5 minutes, (g) a non-zero amount less than 2 minutes, (h) a non-zero amount less than 1 minute, and (i) a time required to pass the preparation through a device housing the primary amine-bearing surface.

5. The method of claim 1, wherein the surface comprises one or more moieties selected from the group consisting of ethylenediamine, tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, and a polyallylamine.

6. The method of claim 1, wherein the surface is a particle, a membrane, or a monolith.

7. The method of claim 1, wherein the contacting step is performed by an operation selected from the group consisting of (a) dispersing primary or secondary amine-bearing particles in the

preparation, (b) passing the preparation through a device in fluid contact with the primary amine-bearing surface, (c) recirculating the preparation through a device in providing fluid contact with the primary amine-bearing surface, and combinations thereof.

8. The method of claim 1, wherein the removing is by sedimentation, filtration, flotation, entrapment in a magnetic field, or entrapment on an oppositely charged surface.

9. The method of claim 1, wherein the surface comprises ethylenediamine.