temperatures facilitate the disruption of the envelope. For example, typical conditions used in accordance with the World Health Organization (WHO) guidelines are 0.3% tri(n-butyl) phosphate (TNBP) and 1% polysorbate 80 sorbitan monooleate (TWEEN® 80) incubated at 24°C for a minimum of 6 hours, or 0.3% TNBP and 1% polyoxyethylene octyl phenyl ether (TRITON® X-100) incubated at 24°C for a minimum of 4 hours. See, e.g., WHO Technical Report, Annex 4 Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products," Series No. 924, p 151-224, (2004).

[006] Incubating the solvent/detergent mixture to a temperature over 20°C has several drawbacks. First, inactivation at higher temperatures is time consuming since pre-heating the mixture to temperatures above 20°C adds about two to about six hours to the overall processing time. Second, incubation at this higher temperature, in conjunction with the agitation necessary to facilitate uniform heating, enhances protein aggregate formation resulting in a reduction in the yield of active and/or useful protein. Exposure to temperatures above 20°C also increases a protein's susceptibility to degradation. The present specification discloses a novel S/D method for inactivating contagious pathogenic viruses from a sample that addresses these drawbacks. The method disclosed herein uses incubation times below two hours and incubation temperatures below 20°C. Decreasing the incubation time reduces the overall time necessary to process and/or manufacture a protein obtained through recombinant production or biological fluid purification. In addition, decreasing both the time and temperature result in improved protein yields by reducing its susceptibility to aggregation and degradation.

SUMMARY

[007] Thus, embodimentaspects of the present specification disclose methods of inactivating a lipid-coat containing virus, the methods comprising the steps of a) providing a fluid comprising a protein having an activity; b) mixing an organic solvent and a surfactant with the fluid, thereby creating a mixture; and c) incubating the mixture for no more than about 120 minutes; wherein both steps (b) and (c) are performed at a temperature of no higher than about 20°C; wherein the mixture after incubation is essentially free of a viable lipid-coat containing virus; and wherein the protein after incubation has an activity of at least 25% of the activity provided in step (a). The fluid can be a cell lysate, a cell supernatant, an elution from a previous purification step, or a biological fluid. The protein can be obtained through recombinant production using a cell line or by purification from a biological fluid. The organic solvent can be an ether, an alcohol, or an alkylphosphate like a dialkylphosphate or a trialkylphosphate. The surfactant can be an ionic surfactant like an anion surfactant or cationic surfactant, a zwitterionic (amphoteric) surfactant, or a non-ionic surfactant. The method may, or may not, further comprises a step of removing the solvent, surfactant and/or the non-viable virus from the mixture after step (c).

[008] Other embodimentaspects of the present specification disclose a protein essentially free of a lipid-coat containing virus obtained from a method comprising the steps of: a) providing a fluid comprising a protein having an activity; b) mixing an organic solvent and a surfactant with the fluid, thereby creating a mixture; and c) incubating the mixture for no more than about 120 minutes; wherein both steps (b) and (c) are

performed at a temperature of no higher than about 20°C; wherein the mixture after incubation is essentially free of a viable lipid-coat containing virus; and wherein the protein after incubation has an activity of at least 25% of the activity provided in step (a).

[009] Yet other embodimentaspects of the present specification disclose methods of inactivating a lipid-coat containing virus, the methods comprising the steps of: a) providing a fluid comprising a Factor VIII having an activity; b) mixing an organic solvent and a surfactant with the fluid, thereby creating a mixture; and c) incubating the mixture for no more than about 120 minutes; wherein both steps (b) and (c) are performed at a temperature of no higher than about 20°C; wherein the mixture after incubation is essentially free of a viable lipid-coat containing virus; and wherein the Factor VIII after incubation has an activity of at least 25% of the activity provided in step (a).

[010] Still other embodimentaspects of the present specification disclose a Factor VIII essentially free of a lipid-coat containing virus obtained from a method comprising the steps of: a) obtaining a fluid comprising a Factor VIII having an activity; b) mixing an organic solvent and a surfactant with the fluid, thereby creating a mixture; and c) incubating the mixture for no more than about 120 minutes; wherein both steps (b) and (c) are performed at a temperature of no higher than about 20°C; wherein the mixture after incubation is essentially free of a viable lipid-coat containing virus; and wherein the Factor VIII after incubation has an activity of at least 25% of the activity provided in step (a).

[010a] One aspect of the invention relates to a method of inactivating a lipid-coat containing virus, the method comprising the steps of: a) providing a fluid comprising a Factor VIII having an activity; b) mixing tri(n-butyl) phosphate, polyoxyethylene octyl phenyl ether, and polysorbate 80 sorbitan monooleate with the fluid, thereby creating a mixture; wherein the final concentration of the tri(n-butyl) phosphate is from about 0.1% (v/v) to about 5.0% (v/v), the final concentration of the polyoxyethylene octyl phenyl ether is from about 0.5% (v/v) to about 10.0% (v/v), and the final concentration of the polysorbate 80 sorbitan monooleate is from about 0.1% (v/v) to about 5.0% (v/v)and c) incubating the mixture for no more than 120 minutes; wherein both steps (b) and (c) are performed at a temperature from between 2°C and 8°C; and wherein the protein after incubation has an activity of at least 25%, at least 50% or at least 75% of the activity provided in step (a).

[010b] Another aspect of the invention relates to a method of inactivating a lipid-coat containing virus, the method comprising the steps of: a) providing a fluid comprising a Factor VIII having an activity; b) mixing tri(n-butyl) phosphate, polyoxyethylene octyl phenyl ether, and polysorbate 80 sorbitan monooleate with the fluid, thereby creating a mixture wherein the concentration of tri(n-butyl) phosphate is 0.3% (v/v), the concentration of polyoxyethylene octyl phenyl ether is 1.0% (v/v), and the concentration of polysorbate 80 sorbitan monooleate is 0.3% (v/v) and c) incubating the mixture for no more than 120 minutes; wherein both steps (b) and (c) are performed at a temperature from between 2 °C and 8 °C; and wherein the Factor VIII after incubation has an activity of at least 25% of the activity provided in step (a).

DESCRIPTION

[011] Aspects of the present specification disclose, in part, a lipid-coat containing virus. A complete virus particle, known as a virion, consists of nucleic acid, either DNA or RNA, surrounded by a protective coat of protein called a capsid. Viruses can be grouped into non-enveloped and enveloped viruses. As used herein, the term "lipid-coat containing virus" refers to any virus comprising a membrane or envelope including lipid, such as, *e.g.*, an envelope virus. Enveloped viruses have their capsid enclosed by a lipoprotein membrane, or envelope. This envelope is derived from the host cell as the virus "buds" from its surface and consists mostly of lipids not encoded by the viral genome. Even though it carries molecular determinants for attachment and entry into target cells, and is essential for the infectivity of enveloped viruses, it is not subject to drug resistance or antigenic shift. Enveloped viruses range in size from about 45-55 nm to about 120-200 nm. Lipid-coat containing viruses which can infect mammalian cells include DNA viruses like a herpesviridae virus, a poxviridae virus, or a hepadnaviridae virus; RNA viruses like a flaviviridae virus, a togaviridae virus, a coronaviridae virus, a deltavirus virus, an orthomyxoviridae virus, a paramyxoviridae virus, a rhabdoviridae virus, a bunyaviridae virus, or a filoviridae virus; and reverse transcribing viruses like a retroviridae virus or a hepadnaviridae virus. Non-limiting examples of lipid-coat containing viruses include a human immunodeficiency virus, a sindbis virus, a herpes simplex virus, a pseudorabies virus, a sendai virus, a vesicular stomatitis virus, a West Nile virus, a bovine viral diarrhea virus, a corona virus, an equine arthritis virus, a severe acute respiratory syndrome virus, Moloney murine leukemia virus, or a vaccinia virus.