[0009] Whereas the inactivation of lipid-enveloped viruses can be performed very effectively by a solvent/detergent (S/D) treatment approach, the inactivation or removal of non-lipid-enveloped model viruses (NLEV's) can be challenging due to their small size and physical stability.

[0010] Thus there exists a need in the art to develop methods to efficiently inactivate or remove non-lipid enveloped viruses during the purification of VWF.

SUMMARY

[0011] The present disclosure provides an efficient method for purifying VWF for increased removal of non-lipid enveloped viruses. The present disclosure provides a novel method of purifying VWF for increased removal of NLEV's by performing the product loading step and the wash step of the purification process at a high pH.

[0012] One method known in the art for purifying polypeptides from NLEV's involves the use of nanofiltration. The principle behind efficient separation of protein and virus using nanofiltration exploits the size difference between the polypeptide and the virus; efficient separation requires the polypeptide to have an effective size smaller that the virus, which allows the polypeptide to pass through the pores of the nanofilter while the virus is retained. If the polypeptide and virus are of a comparable size relative to each other, however, separation is problematic because either the polypeptide and virus both pass through the nanofilter pores or neither do. The methods disclosed herein overcome this problem by using a cation exchange resin rather than nanofiltration and loading and/or washing the resin at a sufficiently high pH to separate the polypeptide from the virus.

[0013] Without being bound by theory, the methods disclosed herein are useful for improved removal of NLEV from polypeptide solutions wherein the polypeptide is of a certain size and/or conformation. A polypeptide of a sufficiently large size is likely to have localized charge characteristics at or above the isoelectric point of the polypeptide, *i.e.*, regions of the polypeptide can maintain localized positive or negative charges, thereby allowing the polypeptide to adsorb to the column resin while the virus flows through. This uneven charge distribution over the length of a polypeptide allows the polypeptide to remain attached to the resin despite loading and/or washing of the resin at a high pH.

[0014] The disclosure provides a method for removing a non-lipid enveloped virus from a protein-containing solution comprising loading a protein in the solution onto a cation

exchange resin, and washing the resin with a buffer at a pH higher than the isoelectric point of the protein to elute the virus.

[0014a] In one aspect, there is provided a method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the protein-containing solution to a cation exchange resin at a pH of at least 1.6 units above the isoelectric point of a protein in the protein-containing solution; and

washing the cation exchange resin with a wash buffer to form an eluate, said wash buffer having a pH that is lower than the pH of the protein-containing solution applied to the cation exchange resin but having a pH higher than the isoelectric point of the protein, wherein the protein in the protein-containing solution has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

[0014b] In one aspect, there is provided a method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the protein-containing solution to a cation exchange resin;

washing the cation exchange resin with a first wash buffer having a pH higher than the pH of the protein-containing solution applied to the cation exchange resin and having a pH higher than the isoelectric point of the protein; and

washing the cation exchange resin with a second wash buffer to form an eluate, said eluate having a pH that is equal to or lower than the first wash buffer, wherein the protein in the protein-containing solution has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

[0014c] In one aspect, there is provided a method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the solution to a cation exchange resin at a pH higher than the isoelectric point of the protein in the protein-containing solution; and

washing the cation exchange resin with a first wash buffer at a pH higher than the isoelectric point of the protein in the protein-containing solution applied to the cation exchange resin; and

washing the cation exchange resin with a second wash buffer to form an eluate, said eluate having a pH that is equal to or lower than the pH of the first wash buffer, wherein the protein in the protein-containing solution has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

[0014d] In one aspect, there is provided a method for removing a non-lipid enveloped virus from a von Willebrand Factor (VWF)-containing solution comprising:

applying the VWF-containing solution to a cation exchange resin at a pH of at least 1.6 units above the isoelectric point of VWF; and

washing the cation exchange resin with a first wash buffer to form an eluate, said wash buffer having a pH that is equal to or lower than the pH of the VWF-containing solution applied to the cation exchange resin and having a pH higher than the isoelectric point of the protein, and whereby the non-lipid enveloped virus is removed from the VWF-containing solution.

[0014e] In one aspect, there is provided a method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the protein-containing solution to a cation exchange resin, wherein the pH of the protein-containing solution is at least 2.0 pH units or more above the isoelectric point of a protein in the protein-containing solution; and

washing the cation exchange resin with a wash buffer to form an eluate, said wash buffer having a pH that is equal to or lower than the pH of the solution applied to the cation exchange resin and having a pH higher than the isoelectric point of the protein, wherein the protein has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

[0014f] In one embodiment, the protein is loaded onto the resin in a buffer at a pH higher than that of the isoelectric point of the protein to elute the virus. In another embodiment, the protein is loaded onto the resin in a buffer that is not the buffer used in the wash step, and the resin is subsequently washed with the buffer that is at a pH higher than an isoelectric point of the protein.

[0015] In one embodiment, a method for removing a non-lipid enveloped virus from a protein-containing solution is provided comprising applying the solution to a cation exchange resin at a pH higher than the isoelectric point of the protein, and washing the cation exchange resin with a first wash buffer to form an eluate, said first wash buffer having a pH that is equal to or lower than the solution applied to the cation exchange resin.

[0016] In one embodiment, the pH of the solution is about 1 pH unit above the isoelectric point of the protein. In other embodiments, the pH of the solution is about 1.1, or about 1.2, or about 1.3, or about 1.4, or about 1.5, or about 1.6, or about 1.7, or about 1.8, or about 1.9, or about 2.0, or about 2.1, or about 2.2, or about 2.3, or about 2.4, or about 2.5, or about 2.6, or about 2.7, or about 2.8, or about 2.9, or about 3.0, or about 3.1, or about 3.2, or about 3.3, or about 3.4, or about 3.5, or about 3.6, or about 3.7, or about 3.8, or about 3.9, or about 4.0, or about 4.1, or about 4.2, or about 4.3, or about 4.4, or about 4.5, or about 4.6, or about 4.7, or about 4.8, or about 4.9, or about 5.0, or about 5.1, or about 5.2, or about 5.3, or about 5.4, or about 5.5, or about 5.6, or about 5.7, or about 5.8, or about 5.9, or about 6.0 or more pH units or more above the isoelectric point of the protein. In these embodiments, the pH is greater than about 7. In a related embodiment, the pH of the protein-containing solution is about 7.0. In other embodiments, the pH of the protein-containing solution is about 7.1, or about 7.2, or about 7.3, or about 7.4, or about 7.5, or about 7.6, or about 7.7, or about 7.8, or about 7.9, or about 8.0, or about 8.1, or about 8.2, or about 8.3, or about 8.4, or about 8.5, or about 8.6, or about 8.7, or about 8.8, or about 8.9, or about 9.0, or about 9.1, or about 9.2, or about 9.3, or about 9.4, or about 9.5, or about 9.6, or about 9.7, or about 9.8, or about 9.9, or about 10.0, or about 10.1, or about 10.2, or about 10.3, or about 10.4, or about 10.5, or about 10.6, or about 10.7, or about 10.8, or about 10.9, or about 11.0, or about 11.1, or about 11.2, or about 11.3, or about 11.4, or about 11.5, or about 11.6, or about 11.7, or about 11.8, or about 11.9, or about 12.0, or about 12.1, or about 12.2, or about 12.3, or about 12.4, or about 12.5, or about 12.6, or about 12.7, or about 12.8, or about 12.9, or about 13.0 or higher.

[0017] In another embodiment, a method is provided for removing a non-lipid enveloped virus from a protein-containing solution comprising applying the solution to a cation exchange resin, washing the cation exchange resin with a first wash buffer at a pH higher than the pH of the solution applied to the cation exchange resin, and washing the cation exchange resin with a second wash buffer to form an eluate, said first eluant having a pH that is equal to or lower than the first wash buffer. In one embodiment, the pH of the first wash buffer is about 1 pH unit above the pH of the solution applied to the cation exchange resin. In other embodiments, the pH of the first wash buffer is about 0.1, or about 0.2, or about 0.3, or about 0.4, or about 0.5, or about 0.6, or about 0.7, or about 0.8, or about 0.9, or about 1.1, or about 1.2, or about 1.3, or about 1.4, or about 1.5, or about 1.6, or about 1.7, or about 1.8, or about 1.9, or about 2.0, or about 2.1, or about 2.2, or about 2.3, or about 2.4, or about 2.5, or about 2.6, or about 2.7, or about 2.8, or about 2.9, or about 3.0, or about 3.1, or about 3.2, or about 3.3, or about 3.4, or about 3.5, or about 3.6, or about 3.7, or about 3.8, or about 3.9, or about 4.0, or about 4.1, or about 4.2, or about 4.3, or about 4.4, or about 4.5, or about 4.6, or about 4.7, or about 4.8, or about 4.9, or about 5.0, or about 5.1, or about 5.2, or about 5.3, or about 5.4, or about 5.5, or about 5.6, or about 5.7, or about 5.8, or about 5.9, or about 6.0, or about 6.1, or about 6.2, or about 6.3, or about 6.4, or about 6.5, or about 6.6, or about 6.7, or about 6.8. or about 6.9, or about 7.0, or about 7.1, or about 7.2, or about 7.3, or about 7.4, or about 7.5, or about 7.6, or about 7.7, or about 7.8, or about 7.9, or about 8 or about 8.1, or about 8.2, or about 8.3, or about 8.4, or about 8.5, or about 8.6, or about 8.7, or about 8.8, or about 8.9, or about 9, or about 9.1, or about 9.2, or about 9.3, or about 9.4, or about 9.5, or about 9.6, or about 9.7, or about 9.8, or about 9.9, or about 10 or more pH units or more above the isoelectric point of the protein. In these embodiments, the pH of the first wash buffer is greater than about 7. In other embodiments, the pH of the first wash buffer is about 7.1, or about 7.2, or about 7.3, or about 7.4, or about 7.5, or about 7.6, or about 7.7, or about 7.8, or about 7.9, or about 8.0, or about 8.1, or about 8.2, or about 8.3, or about 8.4, or about 8.5, or about 8.6, or about 8.7, or about 8.8, or about 8.9, or about 9.0, or about 9.1, or about 9.2, or about 9.3, or about 9.4, or about 9.5, or about 9.6, or about 9.7, or about 9.8, or about 9.9, or about 10.0, or about 10.1, or about 10.2, or about 10.3, or about 10.4, or about 10.5, or about 10.6, or about 10.7, or about 10.8, or about 10.9, or about 11.0 or higher.

[0018] In an embodiment, the protein in the solution is a polypeptide having a molecular mass of at least about 150 kilodaltons. In various embodiments, the protein in the solution is a polypeptide having a molecular mass of at least about 175 kilodaltons, or about 180

kilodaltons, or about 190 kilodaltons, or about 200 kilodaltons, or about 210 kilodaltons, or about 220 kilodaltons, or about 230 kilodaltons, or about 240 kilodaltons, or about 250 kilodaltons, or about 260 kilodaltons, or about 270 kilodaltons, or about 280 kilodaltons, or about 290 kilodaltons, or about 300 kilodaltons, or about 310 kilodaltons, or about 320 kilodaltons, or about 330 kilodaltons, or about 340 kilodaltons, or about 350 kilodaltons, or about 360 kilodaltons, or about 370 kilodaltons, or about 380 kilodaltons, or about 390 kilodaltons, or about 400 kilodaltons, or about 410 kilodaltons, or about 420 kilodaltons, or about 430 kilodaltons, or about 440 kilodaltons, or about 450 kilodaltons, or about 460 kilodaltons, or about 470 kilodaltons, or about 480 kilodaltons, or about 490 kilodaltons, or about 500 kilodaltons or more. As described herein, polypeptides also comprise multimeric structures and such multimeric structures, in various embodiments, have a molecular mass of at least about 500 kilodaltons. In related embodiments, the multimeric structures have a molecular mass of at least about 510, or about 520, or about 530, or about 540, or about 550, or about 560, or about 570, or about 580, or about 590, or about 600, or about 610, or about 620, or about 630, or about 640, or about 650, or about 660, or about 670, or about 680, or about 690, or about 700, or about 710, or about 720, or about 730, or about 740, or about 750, or about 760, or about 770, or about 780, or about 790, or about 800, or about 810, or about 820, or about 830, or about 840, or about 850, or about 860, or about 870, or about 880, or about 890, or about 900, or about 910, or about 920, or about 930, or about 940, or about 950, or about 960, or about 970, or about 980, or about 990 kilodaltons, or about 1 megadalton, or about 1.1 megadaltons, or about 1.2 megadaltons, or about 1.3 megadaltons, or about 1.4 megadaltons, or about 1.5 megadaltons, or about 1.6 megadaltons, or about 1.7 megadaltons, or about 1.8 megadaltons, or about 1.9 megadaltons, or about 2.0 megadaltons, or about 2.1 megadaltons, or about 2.2 megadaltons, or about 2.3 megadaltons, or about 2.4 megadaltons, or about 2.5 megadaltons, or about 2.6 megadaltons, or about 2.7 megadaltons, or about 2.8 megadaltons, or about 2.9 megadaltons, or about 3.0 megadaltons, or about 3.1 megadaltons, or about 3.2 megadaltons, or about 3.3 megadaltons, or about 3.4 megadaltons, or about 3.5 megadaltons, or about 3.6 megadaltons, or about 3.7 megadaltons, or about 3.8 megadaltons, or about 3.9 megadaltons, or about 4.0 megadaltons, or about 4.1 megadaltons, or about 4.2 megadaltons, or about 4.3 megadaltons, or about 4.4 megadaltons, or about 4.5 megadaltons, or about 4.6 megadaltons, or about 4.7 megadaltons, or about 4.8 megadaltons, or about 4.9 megadaltons, or about 5.0 megadaltons or more.

[0019] In some embodiments, the cation exchange resin has a negatively charged group selected from the group consisting of carboxymethyl (CM), sulphoalkyl (SP, SE), sulphate and methylsulfonate (S) as well as any other negatively charged ligand.

[0020] In a further embodiment, the protein is a blood coagulation protein. In various embodiments, the blood coagulation protein is selected from the group consisting of Factor VIII, von Willebrand factor, FI (Fibrinogen), FV (Proaccelerin), FXI (plasma-thromboplastin antecedent), and FXIII (fibrin stabilizing factor).

[0021] In an embodiment, a method for removing a non-lipid enveloped virus from a von Willebrand (VWF)-containing solution is provided comprising applying the solution to a cation exchange resin at a pH higher than the isoelectric point of the protein and washing the cation exchange resin with a first wash buffer to form an eluate, said first wash buffer having a pH that is equal to or lower than the solution applied to the cation exchange resin.

[0022] In another embodiment, a method for removing a non-lipid enveloped virus from a VWF-containing solution is provided comprising applying the solution to a cation exchange resin, washing the cation exchange resin with a first wash buffer at a pH higher than the pH of the solution applied to the cation exchange resin and washing the cation exchange resin with a second wash buffer to form an eluate, said first eluant having a pH that is equal to or lower than the first wash buffer.

[0023] In a further embodiment, a method for removing a non-lipid enveloped virus from a VWF-containing solution comprising applying the solution to a cation exchange resin at a pH higher than the isoelectric point of the protein and washing the cation exchange resin with a first wash buffer at a pH higher than the isoelectric point of the protein applied to the cation exchange resin; and washing the cation exchange resin with a second wash buffer to form an eluate, said first eluant having a pH that is equal to or lower than the first wash buffer.

BRIEF DESCRIPTION OF THE FIGURES

[0024] **Figure 1** shows the result of an SDS-PAGE separation followed by silver staining (**A**) and Western Blot (**B**) analysis for residual rFVIII.

[0025] **Figure 2** shows the stained gel of the UNO S runs with MMV and REO virus spiked samples.

CLAIMS:

1. A method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the protein-containing solution to a cation exchange resin at a pH of at least 1.6 units above the isoelectric point of a protein in the protein-containing solution; and

washing the cation exchange resin with a wash buffer to form an eluate, said wash buffer having a pH that is lower than the pH of the protein-containing solution applied to the cation exchange resin but having a pH higher than the isoelectric point of the protein, wherein the protein in the protein-containing solution has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

2. The method of claim 1 wherein the protein-containing solution applied to the cation exchange resin is at least 1.8 pH units above the isoelectric point of the protein in the protein-containing solution.

3. A method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the protein-containing solution to a cation exchange resin;

washing the cation exchange resin with a first wash buffer having a pH higher than the pH of the protein-containing solution applied to the cation exchange resin and having a pH higher than the isoelectric point of the protein; and

washing the cation exchange resin with a second wash buffer to form an eluate, said eluate having a pH that is equal to or lower than the first wash buffer, wherein the protein in the protein-containing solution has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

4. The method of claim 3 wherein the pH of the first wash buffer is at least 1 pH unit above the isoelectric point of the protein in the protein-containing solution applied to the cation exchange resin.

5. A method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the solution to a cation exchange resin at a pH higher than the isoelectric point of the protein in the protein-containing solution; and

washing the cation exchange resin with a first wash buffer at a pH higher than the isoelectric point of the protein in the protein-containing solution applied to the cation exchange resin; and

washing the cation exchange resin with a second wash buffer to form an eluate, said eluate having a pH that is equal to or lower than the pH of the first wash buffer, wherein the protein in the protein-containing solution has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

6. The method of claim 5 wherein the protein-containing solution applied to the cation exchange resin is at least 1 pH unit above the isoelectric point of the protein in the protein-containing solution.

7. The method of claim 5 wherein the pH of the first wash buffer is at least 1 pH unit above the pH of the protein-containing solution applied to the cation exchange resin.

8. The method of any one of claims 2, 4, 6 and 7 wherein the pH of the protein-containing solution applied to the cation exchange resin is greater than pH 7.0.

9. The method of any one of claims 1-8, wherein the cation exchange resin has a negatively charged group selected from the group consisting of carboxymethyl (CM), sulphoalkyl (SP, SE), sulfated esters of cellulose, heparin and methylsulfonate (S).

10. The method of any one of claims 1-9, wherein the protein in the protein-containing solution is a blood coagulation protein.

11. The method of claim 10, wherein the blood coagulation protein is selected from the group consisting of Factor VIII and von Willebrand factor.

12. A method for removing a non-lipid enveloped virus from a von Willebrand Factor (VWF)-containing solution comprising:

applying the VWF-containing solution to a cation exchange resin at a pH of at least 1.6 units above the isoelectric point of VWF; and

washing the cation exchange resin with a first wash buffer to form an eluate, said wash buffer having a pH that is equal to or lower than the pH of the VWF-containing solution applied to the cation exchange resin and having a pH higher than the isoelectric point of the protein, and whereby the non-lipid enveloped virus is removed from the VWF-containing solution.

13. The method of claim 3, wherein the protein is von Willebrand Factor (VWF).

14. The method of claim 5, wherein the protein is von Willebrand Factor (VWF).

15. The method of claim 1, wherein the protein-containing solution applied to the cation exchange resin is at least 2 pH units above the isoelectric point of the protein in the protein-containing solution.

16. A method for removing a non-lipid enveloped virus from a protein-containing solution comprising:

applying the protein-containing solution to a cation exchange resin, wherein the pH of the protein-containing solution is at least 2.0 pH units or more above the isoelectric point of a protein in the protein-containing solution; and

washing the cation exchange resin with a wash buffer to form an eluate, said wash buffer having a pH that is equal to or lower than the pH of the solution applied to the cation exchange resin and having a pH higher than the isoelectric point of the protein, wherein the protein has a molecular mass of at least about 150 kilodaltons, and whereby the non-lipid enveloped virus is removed from the protein-containing solution.

17. The method of claim 16, wherein the pH of the protein containing-containing solution and the pH of the wash buffer is about 9.0.