materials, and may variously combine charges with hydrophobic groups, metal affinity, groups, or chemical groups that favor formation of hydrogen bonds. They are operated under different chemical conditions depending on the nature of the secondary functionalities, but they are still operated in bind-elute and flow-through mode.

[0004] A small minority of multimodal materials have been described that combine charge with a physical functionality, for example, where a charged material also has the ability to sort species according to their size. One such example employs variable size exclusion functionality in combination with an electropositive surface [Hunter, A., *J. Chromatogr. A* 897:65-80 (2000); Hunter, A., *J. Chromatogr. A* 897:81-97 (2000); Hunter, A., *J. Chromatogr. A* 930:79-93 (2000); Hunter, A., *J. Chromatogr. A* 971:105-116 (2000)]. The method generally involves entry of proteins into particle pores in a size-dependent manner while exhibiting codependence on the charge on the protein, as well as the buffer conditions. Like other charged materials for fractionation, the material described by Hunter *et al* is described exclusively for bind-elute and flow-through applications.

SUMMARY OF THE INVENTION

[0005] Methods, materials, and kits are provided for the purification of virus particles. In one aspect, the present invention refers to a method of purifying a sample comprising a desired virus, the method comprising the steps of (i) providing a packed chromatographic column comprising negatively charged porous particles, (ii) equilibrating the packed chromatographic column with an equilibration buffer to a condition to which the desired virus in the sample is to elute, the condition comprising a pH in a range of 4 to 9 and a conductivity in a range of 0.1 mS/cm to 30 mS/cm, (iii) contacting the sample with the packed chromatographic column wherein a volume of the sample is less than or equal to the void volume of the packed chromatographic column, and (iv) eluting the desired virus from the packed chromatographic column, wherein the desired virus is in a purer state and in the condition to which the packed chromatographic column was equilibrated. In certain ~~embodiments~~examples, ~~the invention provides~~disclosed herein are also methods for purifying a sample containing a desired species of virus including the steps of (i) providing a packed chromatographic column comprising negatively charged porous particles, (ii) equilibrating the packed chromatographic column to the conditions to which the desired virus in the sample is to elute, (iii) contacting the sample with the packed chromatographic column such that the sample volume applied to the packed chromatographic column is less than or equal to the interparticle space of the negatively charged porous particles within the packed chromatographic column, and (iv) eluting the desired virus from the packed chromatographic column, where the desired virus is in a purer state and in the conditions to which the packed chromatographic column was equilibrated.

DETAILED DESCRIPTION OF THE INVENTION

[0006] In certain embodiments, the present invention relates to a column packed with electronegative porous particles in which a virus may be substantially restricted to the space between particles (*i.e.* the inter-particle or void space) by the force of electrostatic repulsion, independently from or in combination with steric rejection from the pores, or solely by steric

derivatives thereof. In certain embodiments, the invention provides organic molecules of the formula R-CO-NH-CO-NH_2 or R-CO-NH-CO-NH-CO-R' or R' R"NH-CO-NR"'R"" where the relevant "R-groups" may be H or any organic moiety.

[0096] "**Virus**" or "**virion**" refers to an ultramicroscopic (roughly 20 to 300 nm in diameter), metabolically inert, infectious agent that replicates only within the cells of living hosts, mainly bacteria, plants, and animals: composed of an RNA or DNA core, a protein coat, and, in more complex types, a surrounding envelope. Examples include lipid-enveloped viruses, non-enveloped viruses, bacteriophages, pseudovirions, and virus-like particles.

[0097] In certain embodiments, a virus can be eluted in the same buffer to which the column is equilibrated prior to sample application. In such embodiments the invention can provide an advantage that it can be used for the purpose of exchanging the buffer in which the sample resides, from its initial formulation to a formulation more suitable for subsequent processing by other methods. While the composition of buffer in which the sample initially resides is unrestricted except by the need to avoid damaging the virus, the composition of the column equilibration buffer must be within a range that does not create positive charge on the virus at a level sufficient to cause a substantial proportion of the virus to bind to the negatively charged particles. Appropriate equilibration conditions vary according to the choice of negatively charged porous particles and the particular features of specific virus. In general, column equilibration conditions may range from but are not limited to conductivity values of less than 0.1 mS/cm to more than 30 mS/cm, and pH values from less than 4.0 to greater than 9.0.

[0098] In certain embodiments where it is an objective of the technique to reduce contaminant levels, it will be advantageous to employ the lowest pH and/or the lowest conductivity that does not cause the virus to bind substantially to the negatively charged particles. It will be understood that the guidelines of lowest pH and/or lowest conductivity may be moderated according to the solubility and stability requirements of the ~~antibody~~virus.

[0099] In certain embodiments, where the electronegative particles bear additional chemical functionalities, it may be necessary or beneficial to employ raise pH values or include additives that modulate the interactivity of the virus with the surface of the particles in order to practice the method. The simplest such variant may be to add NaCl or other salts. The most effective level can be determined experimentally by evaluating different increments of NaCl concentration, such as 25 mM, 50 mM, 100 mM, 200 mM etc. Once the effective range is determined, it may be refined with subsequent experiments conducted at finer

Claims

1. A method of purifying a sample comprising a desired virus, the method comprising the steps of (i) providing a packed chromatographic column comprising negatively charged porous particles, (ii) equilibrating the packed chromatographic column with an equilibration buffer to a condition to which the desired virus in the sample is to elute, the condition comprising a pH in a range of 4 to 9 and a conductivity in a range of 0.1 mS/cm to 30 mS/cm, (iii) contacting the sample with the packed chromatographic column wherein a volume of the sample is less than or equal to the void volume of the packed chromatographic column, and (iv) eluting the desired virus from the packed chromatographic column, wherein the desired virus is in a purer state and in the condition to which the packed chromatographic column was equilibrated.

2. The method of claim 1, wherein the desired virus is a lipid-enveloped virus, a non-lipid-enveloped virus, a bacteriophage, a pseudovirus, or a virus-like particle.

3. The method of claim 1, wherein the sample may be unpurified, at an intermediate level of purity, or highly purified.

4. The method of claim 1, wherein the desired virus has been concentrated prior to its application to the column.

5. The method of claim 1, wherein the desired virus has been concentrated by ultrafiltration prior to its application to the packed chromatographic column.

6. The method of claim 1, wherein the sample has been previously exposed to a negatively charged surface or surfaces under conditions whereby the virus is substantially unbound to the surface or surfaces prior to its application to the packed chromatographic column.

7. The method of claim 6, wherein the negatively charged surface or surfaces further comprise secondary reactivities comprising the ability to participate in hydrophobic interactions, metal affinity interaction, or combinations thereof.

8. The method of any one of claims 6 or 7, wherein the negatively charged surface or surfaces comprise a membrane, a monolith, a fiber or plurality of fibers, a particle or plurality of particles, or a compound construction thereof.

9. The method of claim 1, wherein the packed chromatographic column is packed solely with negatively charged porous particles and the volume of the sample is less than 40% of the volume of the packed chromatographic column.

10. The method of claim 1, wherein the condition to which the desired virus in the sample is to elute comprises a pH in a range selected from the group consisting of (1) from 4 to 8, (2) from 4 to 7, and (3) from 4.5 to 6.0.

11. The method of claim 1, wherein the condition to which the desired virus in the sample is to elute comprises a conductivity value in a range of from 0.1 mS/cm to 15 mS/cm.

12. The method of claim 1, wherein the sample comprises a pH in a range of 2 to 10.

13. The method of claim 1, wherein a conductivity value of the sample comprises a range of from a non-zero conductivity up to a conductivity corresponding to a saturated solution of a particular salt or combination of salts.

14. The method of claim 1, wherein the negatively charged porous particles comprise cation exchange particles.

15. The method of claim 1, wherein electronegativity is conferred to the negatively charged porous particles partly by a moiety selected from the group consisting of a carboxyl, a sulfo, or a phosphor moiety.

16. The method of claim 1, further comprising the additional step of contacting the sample with one or more contaminant-dissociating agents prior to the step of contacting the sample with the packed chromatographic column.

17. The method of claim 16, wherein the contaminant-dissociating agent is selected from the group consisting of salts, nonionic organic polymers, organic solvents, surfactants, and chaotropes, prior to the step of contacting the sample with the column.

18. The method of any one of claims 16 or 17, wherein the contaminant-dissociating agent is a nonionic organic polymer selected from the group consisting of polyethylene glycol, polypropylene glycol and polybutylene glycol.

19. The method of claim 18, wherein the nonionic organic polymer has an average molecular weight is in a range of 130 Daltons to 1000 Daltons.

20. The method of any one of claims 16 to 19, wherein the contaminant-dissociating agent is an organic solvent selected from the group consisting of ethylene glycol, propylene glycol, butylene glycol, dimethylsulfoxide, ethanol, and phenoxyethanol.

21. The method of any one of claims 16 to 20, wherein the contaminant-dissociating agent is provided at a concentration in a range of 0.01% to 25% weight/volume.

22. The method of any one of claims 16 to 21, wherein the contaminant-dissociating agent is a surfactant selected from the group consisting of a polysorbate surfactant, polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether, CHAPS, CHAPSO and octyl glucoside.

23. The method of claim 22, wherein the surfactant is provided at a concentration in a range of 0.001% to 1% weight/volume.

24. The method of any one of claims 22 or 23, wherein the surfactant is provided at a concentration in a range of 0.001% to 0.1% weight/volume.

25. The method of claim 1, wherein the desired virus elutes within the void volume.

26. The method of claim 1, wherein at least 90% of the desired virus elutes within the void volume.

27. The method of claim 1, wherein the desired virus is a bacteriophage.

28. The method of claim 1, wherein the condition to which the desired virus in the sample is to elute comprises a pH in a range of 4.5 to 6.0.

29. The method of claim 1, wherein the negatively charged porous particles are selected from the list consisting of UNOsphere S, UNOsphere Rapid S, NUvia cPrime and Capto MMC.

Claims

1. A method of purifying a sample comprising a desired virus, the method comprising the steps of (i) providing a packed chromatographic column comprising negatively charged porous particles, (ii) equilibrating the packed chromatographic column to the conditions to which the desired virus in the sample is to elute, (iii) contacting the sample with the packed chromatographic column such that the sample volume applied is less than or equal to the interparticle space of the negatively charged porous particles within the packed chromatographic column, (iv) eluting the virus protein from the packed chromatographic column, wherein the desired virus is in a purer state and in the conditions to which the packed chromatographic column was equilibrated.

2. The method of claim 1, wherein the desired virus is a lipid-enveloped virus, a non-lipid-enveloped virus, a bacteriophage, a pseudovirus, or a virus-like particle.

4. The method of claim 1, wherein the sample may be unpurified, at an intermediate level of purity, or highly purified.

5. The method of claim 1, wherein the desired virus has been concentrated prior to its application to the column.

6. The method of claim 1, wherein the desired virus has been concentrated by ultrafiltration prior to its application to the packed chromatographic column.

7. The method of claim 1, wherein the sample has been previously exposed to a negatively charged surface or surfaces under conditions whereby the virus is substantially unbound to the surface or surfaces prior to its application to the packed chromatographic column.

8. The method of claim 7, wherein the negatively charged surface or surfaces further comprise secondary reactivities comprising the ability to participate in hydrophobic interactions, metal affinity interaction, or combinations thereof.

9. The method of any one of claims 7 or 8, wherein the negatively charged surface or surfaces comprise a membrane, a monolith, a fiber or plurality of fibers, a particle or plurality of particles, or a compound construction thereof.

10. The method of claim 1, wherein the packed chromatographic column is packed solely with negatively charged porous particles and a sample volume is less than 40% of the volume of the packed chromatographic column.

11. The method of claim 1, wherein the packed chromatographic column is equilibrated to a pH in a range of from about 2 to about 9.

12. The method of claim 1, wherein the packed chromatographic column is equilibrated to a conductivity value in a range of from about 0.1 mS/cm to about 30 mS/cm.

13. The method of claim 1, wherein the packed chromatographic column is equilibrated with a buffer having a pH in a range selected from the group consisting of (1) from about 4 to about 8, (2) from about 4 to about 7, and (3) from about 4.5 to about 6.0.

14. The method of claim 1, wherein the packed chromatographic column is equilibrated to a conductivity value in a range of from about 0.1 mS/cm to about 15 mS/cm.

15. The method of claim 1, wherein the packed chromatographic column is equilibrated to conditions at or close to a sample application condition for use in a subsequent purification step to be performed upon an eluate comprising the desired virus.

16. The method of claim 1, wherein a sample application condition for contacting the sample with the packed chromatographic column comprises a pH in range of from about 2 to about 10.

17. The method of claim 1, wherein a conductivity value of the sample for contacting the sample with the packed chromatographic column comprises a range of from a non-zero conductivity up to a conductivity corresponding to a saturated solution of a particular salt or combination of salts.

18. The method of claim 1, wherein the step of equilibrating the packed chromatographic column comprises applying to the packed chromatographic column an equilibration buffer having a pH in a range of about 4 to about 9.

19. The method of claim 18, wherein a conductivity value of the equilibration buffer comprises a range of from about 0.1mS/cm to about 30 mS/cm.

20. The method of claim 1, wherein the negatively charged porous particles comprise cation exchange particles.

21. The method of claim 1, wherein electronegativity is conferred to the negatively charged porous particles partly by a moiety selected from the group consisting of a carboxyl, a sulfo, or a phosphor moiety.

22. The methods of claim 1, wherein the packed chromatographic column comprises further particles in addition to the negatively charged porous particles.

23. The method of claim 1 or 22, wherein at least one of the negatively charged porous particles, the further particles, or both possess one or more secondary chemical functionalities selected from the group consisting of anion exchange, hydrophobic interactions, hydrogen bonding, pi-pi interactions, and metal chelation.

24. The method of claim 1, further comprising the additional step of contacting the sample with one or more contaminant-dissociating agents prior to the step of contacting the sample with the packed chromatographic column.

25. The method of claim 24, wherein the contaminant-dissociating agent is selected from the group consisting of salts, nonionic organic polymers, organic solvents, surfactants, and chaotropes, prior to the step of contacting the sample with the column.

26. The method of any one of claims 24 or 25, wherein the contaminant-dissociating is a nonionic organic polymer selected from the group consisting of polyethylene glycol, polypropylene glycol and polybutylene glycol.

27. The method of claim 26, wherein the nonionic organic polymer has an average molecular weight is in a range of from about 130 D to about 1000 D.

28. The method of any one of claims 24 to 27, wherein the contaminant-dissociating is an organic solvent selected from the group consisting of ethylene glycol, propylene glycol, butylene glycol, dimethylsulfoxide, ethanol, and phenoxyethanol.

29. The method of any one of claims 24 to 28, wherein the contaminant-dissociating agent is provided at a concentration in a range of from about 0.01% to about 25% weight/volume.

30. The method of any one of claims 24 to 29, wherein the contaminant-dissociating is a surfactant selected from the group consisting of Tween, triton, CHAPS, CHAPSO and octyl glucoside.

31. The method of claim 30, wherein the surfactant is provided at a concentration in a range of from about 0.001% to about 1% weight/volume.

32. The method of any one of claims 30 or 31, wherein the surfactant is provided at a concentration in a range of from about 0.001% to about 0.1% weight/volume.

33. A kit providing for the convenient practice of a method of any one of claims 1-32.