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 consisting of (i) providing a packed chromatographic column comprising negatively charged porous particles comprising a pore size in a range of 10 nm to 100 nm, (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 the total volume of the sample that is contacted with the column consists of a volume that 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 examples, 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

Claims

1. A method of purifying a sample comprising a desired virus, the method consisting of (i) providing a packed chromatographic column comprising negatively charged porous particles comprising a pore size in a range of 10 nm to 100 nm, (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 the total volume of the sample that is contacted with the column consists of a volume that 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.

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.