AFFINITY CHROMATOGRAPHY DEVICES

FIELD

[0001] The present disclosure relates generally to affinity chromatography, and more specifically to chromatography devices containing a multilayered membrane assembly that enables the separation of a targeted protein or antibody from an aqueous mixture.

BACKGROUND

[0002] Chromatographic methods generally are used to separate and/or purify molecules of interest such as proteins, nucleic acids, and polysaccharides from a mixture. Affinity chromatography specifically involves passing the mixture over a matrix having a ligand specific (*i.e.* a specific binding partner) for the molecule of interest bound to it. Upon contacting the ligand, the molecule of interest is bound to the matrix and is therefore retained from the mixture. Affinity chromatography provides certain advantages over other types of chromatography. For example, affinity chromatography provides a purification method that can isolate a target protein from a mixture of the target protein and other biomolecules in a single step in high yield.

[0003] Despite the advantages of current affinity chromatography devices, there exists a need in the art for a chromatography device that can be used at shorter residence times than conventional devices while providing the same binding capacity or better binding capacities than current offerings and that is re-useable.

SUMMARY

[0003a] In one aspect, the present disclosure refers to an affinity chromatography device comprising: a housing; an inlet to permit fluid flow into said housing; first and second flow distributors, said first flow distributor and said second flow distributor being positioned at opposing ends of said housing; an outlet to permit fluid flow out of said housing, and a wound membrane assembly disposed within said housing, said wound membrane assembly comprising: at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; and at least one impermeable layer, wherein at least one of said polytetrafluoroethylene membrane and said inorganic particles has

covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0003b] In another aspect, the present disclosure refers to an affinity chromatography device comprising: a housing; an inlet to permit fluid flow into said housing; first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing; an outlet to permit fluid flow out of said housing, and a wound membrane assembly disposed within said housing, said wound membrane assembly comprising: a first polytetrafluoroethylene membrane containing therein first inorganic particles having a first nominal particle size; a second polytetrafluoroethylene membrane containing therein second inorganic particles having a second nominal particle size; and at least one impermeable layer, wherein said at least one of said first polytetrafluoroethylene membrane, said second polytetrafluoroethylene membrane, said first inorganic particles, and said second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0003c] In another aspect, the present disclosure refers to a method for separating a target protein or antibody from an aqueous mixture comprising: passing an aqueous mixture through a chromatography device comprising: a housing; first and second flow distributors, said first flow distributor and said second flow distributor being positioned at opposing ends of said housing; and a wound membrane assembly disposed within said housing, said wound membrane assembly comprising: at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; and at least one impermeable layer, wherein said at least one of said polytetrafluoroethylene membrane and said inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0003d] In another aspect, the present disclosure refers to a method for separating a target protein or antibody from an aqueous mixture comprising: passing an aqueous mixture through a chromatography device comprising: a housing; first and second flow distributors, said first flow distributor and said second flow distributor being positioned at opposing ends of said housing; and a wound membrane assembly disposed within said housing, wound membrane assembly comprising: a first polytetrafluoroethylene membrane containing therein first inorganic particles having a first nominal particle size; a second

polytetrafluoroethylene membrane containing therein second inorganic particles having a second nominal particle size; and at least one impermeable layer, wherein at least one of said first polytetrafluoroethylene membrane, said second polytetrafluoroethylene membrane, said first inorganic particles, and said second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody

[0003e] In another aspect, the present disclosure refers to a multi-well affinity chromatography device comprising: a plurality of wells; and a wound membrane assembly disposed within each of said wells, said wound membrane assembly comprising: a wound membrane assembly disposed within each of said wells, said wound membrane assembly comprising: at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; and at least one impermeable layer, wherein at least one of said polytetrafluoroethylene membrane and said inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0003f] In another aspect, the present disclosure refers to a multi-well affinity chromatography device comprising: a plurality of wells; and a wound membrane assembly disposed within each of said wells, said wound membrane assembly comprising: a first polytetrafluoroethylene membrane containing therein first inorganic particles having a first nominal particle size; a second polytetrafluoroethylene membrane containing therein second inorganic particles having a second nominal particle size; and at least one impermeable layer, wherein at least one of said first polytetrafluoroethylene membrane, said second polytetrafluoroethylene membrane, said first inorganic particles, and said second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0003g] In another aspect, the present disclosure refers to an affinity chromatography device comprising: a housing; an inlet to permit fluid flow into said housing; first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing; an outlet to permit fluid flow out of said housing, and a wound membrane assembly disposed within said housing, said wound membrane assembly comprising at least one polytetrafluoroethylene membrane containing therein inorganic particles having at

least one nominal particle size, wherein said inorganic particles have both epoxide and aldehyde functional groups attached thereto, and wherein at least one of said epoxide and aldehyde functional groups has bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0003h] In another aspect, the present disclosure refers to an affinity chromatography device comprising: a housing; an inlet to permit fluid flow into said housing; first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing; an outlet to permit fluid flow out of said housing, and a stacked membrane assembly disposed within said housing, said stacked membrane assembly comprising at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; wherein said inorganic particles have both epoxide and aldehyde functional groups attached thereto, and wherein at least one of said epoxide and aldehyde said functional group has bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0004] One example relates to an affinity chromatography device that includes a housing, a first flow distributor and a second flow distributor positioned at opposing ends of the housing, an inlet to permit fluid flow into the housing, an outlet to permit fluid flow out of the housing, and a wound membrane assembly disposed within the housing. The wound membrane assembly includes (1) at least one polymer membrane containing therein inorganic particles having at least one nominal particle size and (2) at least one impermeable layer. The impermeable layer may include at least one thermoplastic polymer in a solid state. At least one polymer membrane and/or the inorganic particles has covalently bound thereto an affinity

ligand that reversibly binds to a targeted protein or antibody. In one example, the inorganic particles may have a single nominal particle size. The impermeable layer may be formed of a thermoplastic polymer in a solid state. In another example, the inorganic particles may have a first nominal particle size and a second nominal particle size. In yet another example, the polymer membrane may include first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size. In one or more example, the first and second inorganic particles are of the same particle type. The inorganic particles have a nominal particle size that may be about 5 microns, about 10 microns, about 15 microns, about 20 microns, about 25 microns and combinations thereof.

[0005] Another example relates to an affinity chromatography device that includes a housing, a first flow distributor and a second flow distributor positioned at opposing ends of the housing, an inlet to permit fluid flow into the housing, an outlet to permit fluid flow out of the housing, and a wound membrane assembly disposed within said housing. The wound membrane assembly includes (1) a first polymer membrane containing therein first inorganic particles having a first nominal particle size, (2) a second polymer membrane containing therein second inorganic particles having a second nominal particle size, and (3) at least one impermeable layer. The impermeable layer may include at least one thermoplastic polymer in a solid state. At least one of the first polymer membrane, the second polymer membrane, and the inorganic particles has covalently bound thereto an affinity ligand that reversibly binds to a targeted protein or antibody. In one example, the first and second inorganic particles may have a single nominal particle size. In another example, the first inorganic particles may have a first nominal particle size and the second inorganic particles may have a second nominal particle size. The first and second nominal particle sizes may be the same or different. Also, the first and second polymer membranes may be the same type or different types. The inorganic particles have a nominal particle size that may be about 5 microns, about 10 microns, about 15 microns, about 20 microns, about 25 microns and combinations thereof.

[0006] A further example relates to a method for separating a target protein or antibody from an aqueous mixture that includes passing an aqueous mixture through a chromatography device that includes a housing, a first flow

distributor and a second flow distributor positioned at opposing ends of the housing, an inlet to permit fluid flow into the housing, an outlet to permit fluid flow out of the housing, and a wound membrane assembly disposed within the housing. The wound membrane assembly includes (1) at least one polymer membrane containing therein inorganic particles having at least one nominal particle size and (2) at least one impermeable layer. The impermeable layer may include at least one thermoplastic polymer in a solid state. At least one polymer membrane and/or the inorganic particles has covalently bound thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0007] Yet another example relates to a method for separating a target protein or antibody from an aqueous mixture that includes passing an aqueous mixture through a chromatography device that includes a housing, a first flow distributor and a second flow distributor positioned at opposing ends of the housing, an inlet to permit fluid flow into the housing, an outlet to permit fluid flow out of the housing, and a wound membrane assembly disposed within the housing. The wound membrane assembly includes (1) a first polymer membrane containing therein first inorganic particles having a first nominal particle size, (2) a second polymer membrane containing therein second inorganic particles having a second nominal particle size, and (3) at least one impermeable layer. The impermeable layer may include at least one thermoplastic polymer in a solid state. At least one of the first polymer membrane, the second polymer membrane, and the inorganic particles has covalently bound thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0008] Another example relates to a multi-well affinity chromatography device that includes a plurality of wells and a wound membrane assembly disposed within at least one of the wells. The wound membrane assembly includes (1) at least one polymer membrane containing therein inorganic particles having at least one nominal particle size and (2) at least one impermeable layer. The impermeable layer may include at least one thermoplastic polymer in a solid state. At least one polymer membrane and/or the inorganic particles has covalently bound thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0009] Yet another example relates to a multi-well affinity chromatography device that includes a plurality of wells and a wound membrane assembly disposed within at least one of the wells. The wound membrane assembly

includes (1) a first polymer membrane containing therein first inorganic particles having a first nominal particle size, (2) a second polymer membrane containing therein second inorganic particles having a second nominal particle size, and (3) at least one impermeable layer. The impermeable layer may include at least one thermoplastic polymer in a solid state. At least one of the first polymer membrane, the second polymer membrane, and the inorganic particles has covalently bound thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[00010] A further example relates to an affinity chromatography device that includes a housing, a first flow distributor and a second flow distributor positioned at opposing ends of the housing, an inlet to permit fluid flow into the housing, an outlet to permit fluid flow out of the housing, and a wound membrane assembly disposed within the housing. The wound membrane includes at least one polymer membrane containing therein inorganic particles having at least one nominal particle size. The organic particles have both epoxide and aldehyde functional groups attached thereto. At least one of the epoxide and aldehyde functional groups has bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[00011] Another example relates to an affinity chromatography device that includes a housing, a first flow distributor and a second flow distributor positioned at opposing ends of the housing, an inlet to permit fluid flow into the housing, an outlet to permit fluid flow out of the housing, and a stacked membrane assembly disposed within the housing. The stacked membrane includes at least one polymer membrane containing therein inorganic particles having at least one nominal particle size. The organic particles have both epoxide and aldehyde functional groups attached thereto. At least one of the epoxide and aldehyde functional groups has bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[00012] The accompanying drawings are included to provide a further understanding of the disclosure and are incorporated in and constitute a part of this specification, illustrate embodiments, and together with the description serve to explain the principles of the disclosure.

WHAT IS CLAIMED IS:

1. An affinity chromatography device comprising:

a housing;

an inlet to permit fluid flow into said housing;

first and second flow distributors, said first flow distributor and said second flow distributor being positioned at opposing ends of said housing;

an outlet to permit fluid flow out of said housing, and

a wound membrane assembly disposed within said housing, said wound membrane assembly comprising:

at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; and

at least one impermeable layer,

wherein at least one of said polytetrafluoroethylene membrane and said inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

2. The affinity chromatography device of claim 1, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

3. The affinity chromatography device of claim 1, wherein said inorganic particles are selected from silica particles, zeolite particles, hydroxyapatite particles, metal oxide particles and combinations thereof.

4. The affinity chromatography device of claim 1, wherein said inorganic particles have a single nominal particle size.

5. The affinity chromatography device of claim 1, wherein said inorganic particles comprise a first nominal particle size and a second nominal particle size.

6. The affinity chromatography device of claim 1, wherein said polytetrafluoroethylene membrane comprises first inorganic particles having a first

nominal particle size and second inorganic particles having a second nominal particle size.

7. The affinity chromatography device of claim 6, wherein said first and second inorganic particles are of the same particle type.

8. The affinity chromatography device of claim 6, wherein said first and second inorganic particles comprise different particle types.

9. The affinity chromatography device of claim 1, wherein said polytetrafluoroethylene membrane is an expanded polytetrafluoroethylene membrane.

10. The affinity chromatography device of claim 1, wherein said affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.

11. The affinity chromatography device of claim 1, wherein said nominal particle size is selected from about 0.1 microns, about 0.5 microns, about 1 micron, about 5 microns, about 10 microns, about 15 microns, about 20 microns and about 25 microns.

12. The affinity chromatography device of claim 1, wherein in cross-section, the membrane assembly comprises said polytetrafluoroethylene membrane in an alternating configuration with said impermeable membrane.

13. The affinity chromatography device of claim 2, wherein said thermoplastic polymer is selected from polypropylene, polyethylene, fluorinated ethylene propylene and combinations thereof.

14. An affinity chromatography device comprising:
a housing;
an inlet to permit fluid flow into said housing;

first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing;

an outlet to permit fluid flow out of said housing, and

a wound membrane assembly disposed within said housing, said wound membrane assembly comprising:

a first polytetrafluoroethylene membrane containing therein first inorganic particles having a first nominal particle size;

a second polytetrafluoroethylene membrane containing therein second inorganic particles having a second nominal particle size; and

at least one impermeable layer,

wherein said at least one of said first polytetrafluoroethylene membrane, said second polytetrafluoroethylene membrane, said first inorganic particles, and said second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

15. The affinity chromatography device of claim 14, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

16. The affinity chromatography device of claim 14, wherein said first and second inorganic particles are selected from silica particles, zeolite particles, hydroxyapatite particles, metal oxide particles and combinations thereof.

17. The affinity chromatography device of claim 14, wherein first and second inorganic particles have a single nominal particle size.

18. The affinity chromatography device of claim 17, wherein said first and second inorganic particles are of the same particle type.

19. The affinity chromatography device of claim 17, wherein said first and second inorganic particles comprise different particle types.

20. The affinity chromatography device of claim 14, wherein first and second inorganic particles have different nominal particle sizes.

21. The affinity chromatography device of claim 20, wherein said first and second inorganic particles are of the same particle type.

22. The affinity chromatography device of claim 20, wherein said first and second inorganic particles comprise different particle types.

23. The affinity chromatography device of claim 14, wherein said first polytetrafluoroethylene membrane comprises first and second inorganic particles having different nominal particle sizes.

24. The affinity chromatography device of claim 14, wherein said second polytetrafluoroethylene membrane further comprises the first inorganic particles having the first nominal particle size.

25. The affinity chromatography device of claim 14, wherein said first polytetrafluoroethylene membrane further comprises third inorganic particles having a third nominal particle size, and

wherein said second polytetrafluoroethylene membrane further comprises fourth inorganic particles having a fourth nominal particle size.

26. The affinity chromatography device of claim 14, wherein said first and second polytetrafluoroethylene r membranes are an expanded polytetrafluoroethylene membrane.

27. The affinity chromatography device of claim 14, wherein said affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.

28. The affinity chromatography device of claim 14, wherein said first nominal particle size and said second nominal particle size are selected from about 0.1 microns, about 0.5 microns, about 1 micron, about 5 microns, about 10 microns, about 15 microns, about 20 microns and about 25 microns.

29. A method for separating a target protein or antibody from an aqueous mixture comprising:

passing an aqueous mixture through a chromatography device comprising:

a housing;

first and second flow distributors, said first flow distributor and said second flow distributor being positioned at opposing ends of said housing; and

a wound membrane assembly disposed within said housing, said wound membrane assembly comprising:

at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; and

at least one impermeable layer,

wherein said at least one of said polytetrafluoroethylene membrane and said inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

30. The method of claim 29, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

31. A method for separating a target protein or antibody from an aqueous mixture comprising:

passing an aqueous mixture through a chromatography device comprising:

a housing;

first and second flow distributors, said first flow distributor and said second flow distributor being positioned at opposing ends of said housing; and

a wound membrane assembly disposed within said housing, wound membrane assembly comprising:

a first polytetrafluoroethylene membrane containing therein first inorganic particles having a first nominal particle size;

a second polytetrafluoroethylene membrane containing therein second inorganic particles having a second nominal particle size; and

at least one impermeable layer,

wherein at least one of said first polytetrafluoroethylene membrane, said second polytetrafluoroethylene membrane, said first inorganic particles, and said

second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

32. The method of claim 31, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

33. A multi-well affinity chromatography device comprising:

a plurality of wells; and

a wound membrane assembly disposed within each of said wells, said wound membrane assembly comprising:

a wound membrane assembly disposed within each of said wells, said wound membrane assembly comprising:

at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size; and

at least one impermeable layer,

wherein at least one of said polytetrafluoroethylene membrane and said inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

34. The multi-wall affinity chromatography device of claim 33, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

35. A multi-well affinity chromatography device comprising:

a plurality of wells; and

a wound membrane assembly disposed within each of said wells, said wound membrane assembly comprising:

a first polytetrafluoroethylene membrane containing therein first inorganic particles having a first nominal particle size;

a second polytetrafluoroethylene membrane containing therein second inorganic particles having a second nominal particle size; and

at least one impermeable layer,

wherein at least one of said first polytetrafluoroethylene membrane, said second polytetrafluoroethylene membrane, said first inorganic particles, and said

second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

36. The multi-wall affinity chromatography device of claim 35, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

37. An affinity chromatography device comprising:

a housing;

an inlet to permit fluid flow into said housing;

first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing;

an outlet to permit fluid flow out of said housing, and

a wound membrane assembly disposed within said housing, said wound membrane assembly comprising at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size,

wherein said inorganic particles have both epoxide and aldehyde functional groups attached thereto, and

wherein at least one of said epoxide and aldehyde functional groups has bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

38. The affinity chromatography device of claim 37, wherein the wound membrane further comprises at least one impermeable layer.

39. The affinity chromatography device of claim 37, wherein said at least one impermeable layer comprises at least one thermoplastic polymer membrane in a solid state.

40. An affinity chromatography device comprising:

a housing;

an inlet to permit fluid flow into said housing;

first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing;

an outlet to permit fluid flow out of said housing, and

a stacked membrane assembly disposed within said housing, said stacked membrane assembly comprising at least one polytetrafluoroethylene membrane containing therein inorganic particles having at least one nominal particle size;

wherein said inorganic particles have both epoxide and aldehyde functional groups attached thereto, and

wherein at least one of said epoxide and aldehyde said functional group has bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

41. The affinity chromatography device of any one of claims 1-28 or 37-40 or the multi-wall affinity chromatography device of any one of claims 33-36, wherein when the affinity ligands comprise Fc binding proteins, the affinity chromatography device is configured to provide a dynamic binding capacity of at least 30 mg/ml at 10% breakthrough at a residence time of 20 seconds or less.

42. The device of claim 41, wherein when the affinity ligands comprise at least one of antibodies, non-Fc binding proteins, or polysaccharides, the device is configured to provide a dynamic binding capacity of at least 0.7 µmol/ml, at 10% breakthrough, and at a residence time of 20 seconds or less.