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

[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 stacked membrane assembly disposed within the housing. The stacked membrane assembly includes two or more polymer membranes that contain therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size. At least one of the polymer membranes, the first inorganic particles, and the second inorganic particles has covalently bonded thereto an affinity ligand that reversibly

binds to a targeted protein or antibody. 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.

[0004a] In one aspect, the present invention provides an affinity chromatography device comprising: a housing; and 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 polymer membrane containing therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size, wherein said at least one of said polymer 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.

[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 the housing. The housing may be a cylindrical housing. The wound membrane assembly includes at least one polymer membrane that contains therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size. At least one of the polymer membrane, the first inorganic particles, and the second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody. 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.

[0006] Yet 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

assembly includes two or more polymer membranes that contain therein inorganic particles having a nominal particle size. At least one of the polymer membranes and the inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody. In at least one example, the polymer membranes are of the same polymer type.

[0006a] In another aspect, the present invention provides 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 single polymer membrane containing therein inorganic particles of the same type having a nominal particle size, wherein said at least one of said polymer membrane and said inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0007] 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. The wound membrane assembly includes a single polymer membrane containing therein inorganic particles having a single nominal particle size. At least

one of the polymer membrane and the inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody. 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. In one example, the polymer membrane is a polytetrafluoroethylene membrane. .

[0008] 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 assembly includes a first polymer membrane containing therein first inorganic particles having a first nominal particle size and a second polymer membrane containing therein second inorganic particles having a second nominal particle size. At least one of the first polymer membrane, the second polymer membrane, and the inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody.

[0008a] In yet another aspect, the present invention provides 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 polymer membrane containing therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size, wherein said at least one of said polymer 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.

[0009] 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 stacked membrane assembly disposed within the housing. The membrane assembly includes two or more polymer membranes that contain therein first inorganic particles having a first nominal particle size second inorganic particles having a second nominal particle size. At least one of the polymer membranes, the first inorganic particles, and the second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody. In one or more example, the first and second inorganic particles are of the same particle type.

[00010] 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 at least one polymer membrane that contains therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size. At least one of the polymer membrane, the first inorganic particles, and the second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody. In one or more example, the first and second inorganic particles are of the same particle type.

[0010a] In yet another aspect, the present invention provides 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: at least one polymer membrane containing therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size, wherein said at least one of said polymer 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.

[00011] Another example relates to a multi-well affinity chromatography device that includes a plurality of wells and a stacked membrane assembly disposed within at least one of the wells. The stacked membrane assembly includes two or more polymer membranes that contain therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size. At least one of the polymer membranes, the first inorganic particles, and the second inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted protein or antibody. In one or more example, the first and second inorganic particles are of the same particle type.

[00012] 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 at least one polymer membrane that contains therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size. At least one of the polymer membrane, the first inorganic particles, and the second inorganic particles has covalently bonded thereto an

affinity ligand that reversibly binds to a targeted protein or antibody. In one or more example, the first and second inorganic particles are of the same particle type.

BRIEF DESCRIPTION OF THE DRAWINGS

[00013] 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; and

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 polymer membrane containing therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size,

wherein said at least one of said polymer 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.

2. The affinity chromatography device of claim 1, wherein said first and second inorganic particles are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.

3. The affinity chromatography device of claim 1, wherein said polymer membranes are polytetrafluoroethylene membranes.

4. 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.

5. The affinity chromatography device of claim 1, wherein said first and second particle sizes are selected 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.

6. The affinity chromatography device of claim 1, wherein said first nominal particle size is different from said second nominal particle size.

7. The affinity chromatography device of claim 1, wherein said first and second inorganic particles are of the same particle type.

8. 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 single polymer membrane containing therein inorganic particles of the same type having a nominal particle size,

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

9. The affinity chromatography device of claim 8, 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.

10. The affinity chromatography device of claim 8, wherein said polymer membranes are polytetrafluoroethylene membranes.

11. The affinity chromatography device of claim 8, wherein said inorganic particles are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.

12. The affinity chromatography device of claim 8, wherein said affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies that specifically bind to other proteins, and heparin

13. 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 polymer membrane containing therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size,

wherein said at least one of said polymer 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.

14. 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:

at least one polymer membrane containing therein first inorganic particles having a first nominal particle size and second inorganic particles having a second nominal particle size,

wherein said at least one of said polymer 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 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.

16. The affinity chromatography device of claim 14, wherein said polymer membranes are polytetrafluoroethylene membranes.

17. The affinity chromatography device of claim 14, wherein said inorganic particles are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.

18. 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 that specifically bind to other proteins, and heparin.