

**AFFINITY CHROMATOGRAPHY DEVICES
CONTAINING A HEAT TREATED FIBRILLATED POLYMER MEMBRANE
AND MANIFOLDS CONTAINING THE SAME**

FIELD

[0001] The present disclosure relates generally to affinity chromatography, and more specifically, to an affinity chromatography device that includes a fibrillated heat treated polymer membrane containing therein spherical particles, a particle size distribution that has a D90/D10 less than or equal to 3, and which enables the separation of a targeted molecule from an aqueous mixture. Manifolds containing multiple affinity chromatography devices and manifolds in a parallel configuration are also disclosed.

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] In one Aspect ("Aspect 1"), an affinity chromatography device includes a fluid inlet, a fluid outlet connected to the fluid inlet, a fibrillated heat treated polymer membrane positioned between the fluid inlet and fluid outlet and containing therein inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns, a housing encompassing the fluid inlet, the fluid outlet

and the fibrillated heat treated polymer membrane, where a particle size distribution has a D90/D10 less than or equal to 3, and where at least one of the fibrillated heat treated polymer membrane and the inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted molecule.

[0005] According to another Aspect ("Aspect 2") further to Aspect 1, where the targeted molecule is a protein, antibody, viral vector, and combinations thereof.

[0006] According to another Aspect ("Aspect 3") further to Aspect 1 or Aspect 2, a hydraulic permeability from about 200 ($\times 10^{-12} \text{ cm}^2$) to about 700 ($\times 10^{-12} \text{ cm}^2$).

[0007] According to another Aspect ("Aspect 4") further to any one of Aspects 1 to 3, the inorganic particles having a spherical shape are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.

[0008] According to another Aspect ("Aspect 5") further to any one of Aspects 1 to 4, where the fibrillated polymer membrane comprises an expanded polytetrafluoroethylene membrane, an expanded modified polytetrafluoroethylene membrane, an expanded tetrafluoroethylene copolymer membrane, or an expanded polyethylene membrane.

[0009] According to another Aspect ("Aspect 6") further to any one of Aspects 1 to 5, where the fibrillated heat treated polymer membrane is an expanded polytetrafluoroethylene membrane.

[00010] According to another Aspect ("Aspect 7") further to any one of Aspects 1 to 6, where the affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.

[00011] According to another Aspect ("Aspect 8") further to any one of Aspects 1 to 7, where the inorganic particles comprise at least a first inorganic particle having a spherical shape and a first nominal particle size and a second inorganic particle having a spherical shape and a second nominal particle size, the first and second nominal particle sizes being different from each other.

[00012] According to another Aspect ("Aspect 9") further to any one of Aspects 1 to 8, where the nominal particle size is selected from about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations thereof.

[00013] According to another Aspect ("Aspect 10") further to any one of Aspects 1 to 9 where the inorganic particles having a spherical shape comprise a blend of 10 micron spherical particles and 20 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00014] According to another Aspect ("Aspect 11") further to any one of Aspects 1 to 9, where the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 10 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00015] According to another Aspect ("Aspect 12") further to any one of Aspects 1 to 9, where the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 20 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00016] According to another Aspect ("Aspect 13") further to any one of Aspects 1 to 12, including a dynamic binding capacity (DBC) of at least 35 mg/ml at a residence time of 20 seconds.

[00017] According to another Aspect ("Aspect 14") further to any one of Aspects 1 to 13, including a cycling durability of at least 100 cycles at an operating pressure less than 0.3 MPa.

[00018] According to another Aspect ("Aspect 15") further to any one of Aspects 1 to 14, where the fibrillated heat treated polymer membrane has a wound configuration.

[00019] According to another Aspect ("Aspect 16") further to any one of Aspects 1 to 15, where the fibrillated polymer membrane has a stacked configuration.

[00020] According to another Aspect ("Aspect 17") further to any one of Aspects 1 to 16, where the fibrillated polymer membrane has a wound configuration, a stacked configuration, and a combination thereof.

[00021] According to another Aspect ("Aspect 18") further to any one of Aspects 1 to 17, where an inner intermediate material is circumferentially positioned on an outer surface of a core and where the fibrillated polymer membrane is circumferentially positioned on the inner intermediate material.

[00022] According to another Aspect ("Aspect 19") further to any one of Aspects 1 to 18, including an outer intermediate material circumferentially positioned on the heat treated fibrillated polymer membrane.

[00023] According to another Aspect ("Aspect 20") further to any one of Aspects 1 to 19, where the inner intermediate material and outer intermediate material are selected from a porous fluoropolymer film, a porous non-fluoropolymer film, a porous non-woven material and a porous woven material.

[00024] According to another Aspect ("Aspect 21") further to any one of Aspects 1 to 20, where at least one of the inner intermediate material and outer intermediate material is a polypropylene non-woven material.

[00025] In one Aspect ("Aspect 22") a manifold includes at least two of the affinity chromatography devices of any one of claims 1 to 21 are arranged in a parallel configuration.

[00026] According to another Aspect ("Aspect 23") further to Aspect 22, where the manifold is enclosed within a housing.

[00027] According to another Aspect ("Aspect 24") where the article of any one of Aspects 1 to 21 is used to separate the targeted substance from a biological sample.

[00028] In one Aspect ("Aspect 25") a device includes a first manifold and a second manifold in a parallel configuration and where each of the first manifold and the second manifold includes a plurality of the affinity chromatography device of any one of Aspects 1 to 21.

[00029] In another Aspect ("Aspect 26") further to Aspect 25, where the first manifold and the second manifold are enclosed within a housing.

[00030] According to one Aspect ("Aspect 27"), an article includes a centrally located core, a fibrillated heat treated polymer membrane containing therein spherical inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns wound around the core, a housing surrounding both the core and the fibrillated heat treated polymer membrane, a first end cap positioned at a first end of the housing, and a second end cap positioned at a second end of the housing, a particle size distribution has a D90/D10 less than 3, and where at least one of the fibrillated heat treated polymer membrane and the spherical inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted molecule.

[00031] According to another Aspect ("Aspect 28") further to Aspect 27, where the targeted molecule is a protein, antibody, viral vector, and combinations thereof.

[00032] According to another Aspect ("Aspect 29") further to Aspect 27 or Aspect 28, including a hydraulic permeability from about 200 ($\times 10^{12} \text{ cm}^2$) to about 700 ($\times 10^{12} \text{ cm}^2$).

[00033] According to another Aspect ("Aspect 30") further to any one of Aspects 27 to 29, where the inorganic particles having a spherical shape are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.

[00034] According to another Aspect ("Aspect 31") further to any one of Aspects 27 to 30, where the fibrillated polymer membrane includes at least one of an expanded polytetrafluoroethylene membrane, an expanded modified polytetrafluoroethylene membrane, an expanded tetrafluoroethylene copolymer membrane, or an expanded polyethylene membrane.

[00035] According to another Aspect ("Aspect 32") further to any one of Aspects 27 to 31, where the fibrillated heat treated polymer membrane is a fibrillated heat treated expanded polytetrafluoroethylene membrane.

[00036] According to another Aspect ("Aspect 33") further to any one of Aspects 27 to 32, where the affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.

[00037] According to another Aspect ("Aspect 34") further to any one of Aspects 27 to 33, where the inorganic particles comprise at least a first inorganic particle having a spherical shape and a first nominal particle size and a second inorganic particle having a spherical shape and a second nominal particle size, and where the first and second nominal particle sizes are different from each other.

[00038] According to another Aspect ("Aspect 35") further to any one of Aspects 27 to 34, where the nominal particle size is selected from about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations thereof.

[00039] According to another Aspect ("Aspect 36") further to any one of Aspects 27 to 35, the inorganic particles having a spherical shape comprise a blend of 10 micron spherical particles and 20 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00040] According to another Aspect ("Aspect 37") further to any one of Aspects 27 to 35, where the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 10 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00041] According to another Aspect ("Aspect 38") further to any one of Aspects 27 to 35, where the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 20 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00042] According to another Aspect ("Aspect 39") further to any one of Aspects 27 to 38, including a dynamic binding capacity (DBC) of at least 35 mg/ml at a residence time of 20 seconds.

[00043] According to another Aspect ("Aspect 40") further to any one of Aspects 27 to 39, including a cycling durability of at least 100 cycles and an operating pressure less than 0.3 MPa.

[00044] According to another Aspect ("Aspect 41") further to any one of Aspects 27 to 40, where an inner intermediate material is circumferentially positioned on an outer surface of a core and the fibrillated heat treated polymer membrane is circumferentially positioned on the inner intermediate material.

[00045] According to another Aspect ("Aspect 42") further to any one of Aspects 27 to 41, including an outer intermediate material circumferentially positioned on the fibrillated heat treated polymer membrane

[00046] According to another Aspect ("Aspect 43") further to any one of Aspects 27 to 42, where the inner intermediate material and the outer intermediate material are selected from a porous fluoropolymer film, a porous non-fluoropolymer film, a porous non-woven material and a porous woven material.

[00047] According to another Aspect ("Aspect 44") further to any one of Aspect 27 to 43, where at least one of the inner intermediate material and the outer intermediate material is a polypropylene non-woven material.

[00048] In one Aspect ("Aspect 45") a manifold includes at least two of the affinity chromatography devices of any one of Aspects 27 to 44 arranged in a parallel configuration.

[00049] According to another Aspect ("Aspect 46") further to Aspect 45, where the manifold is enclosed within a housing.

[00050] In one Aspect ("Aspect 47") a device includes a first manifold and a second manifold in a parallel configuration where each of the first manifold and the second manifold includes at least two of the affinity chromatography device of any one of Aspects 27 to 44.

[00051] According to another Aspect ("Aspect 48") further to Aspect 47, the first manifold and the second manifold are enclosed within a housing.

[00052] According to another Aspect ("Aspect 49") where the article of any one of Aspects 27 to 44 are used to separate the targeted molecule from a fluid stream.

[00053] According to one Aspect ("Aspect 50"), an affinity chromatography device includes a housing, an inlet to permit fluid flow into the 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, a stacked membrane assembly disposed within said housing between the fluid inlet and fluid outlet, said stacked membrane assembly, where the stacked membrane assembly includes two or more fibrillated heat treated polymer membranes in a stacked configuration, where the fibrillated heat treated polymer membrane contains therein inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns, where a particle size distribution has a D90/D10 less than 3, and where at least one of the fibrillated heat treated polymer membrane and the spherical inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted molecule.

[00054] According to another Aspect ("Aspect 51") further to Aspect 50, where the targeted molecule is a protein, antibody, viral vector, and combinations thereof.

[00055] According to another Aspect ("Aspect 52") further to Aspect 50 or Aspect 51, including first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of the housing.

[00056] According to another Aspect ("Aspect 53") further to any one of Aspects 50 to 52 including a hydraulic permeability from about 200 ($\times 10^{-12} \text{ cm}^2$) to about 700 ($\times 10^{-12} \text{ cm}^2$).

[00057] According to another Aspect ("Aspect 54") further to any one of Aspects 50 to 53, where the inorganic particles having a spherical shape are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.

[00058] According to another Aspect ("Aspect 55") further to any one of Aspects 50 to 54, where the fibrillated polymer membrane includes an expanded polytetrafluoroethylene membrane, an expanded modified polytetrafluoroethylene membrane, an expanded tetrafluoroethylene copolymer membrane, or an expanded polyethylene membrane.

[00059] According to another Aspect ("Aspect 56") further to any one of Aspects 50 to 55, where the fibrillated heat treated polymer membrane is a fibrillated heat treated expanded polytetrafluoroethylene membrane.

[00060] According to another Aspect ("Aspect 57") further to any one of Aspects 50 to 56, the affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.

[00061] According to another Aspect ("Aspect 58") further to any one of Aspects 50 to 57, where the inorganic particles comprise at least a first inorganic particle having a spherical shape and a first nominal particle size and a second inorganic particle having a spherical shape and a second nominal particle size, where the first and second nominal particle sizes are different from each other.

[00062] According to another Aspect ("Aspect 59") further to any one of Aspects 50 to 58, where the nominal particle size is selected from about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations thereof.

[00063] According to another Aspect ("Aspect 60") further to any one of Aspects 50 to 59, where the inorganic particles having a spherical shape comprises a blend of 10 micron spherical particles and 20 micron spherical particles, and where the blend is from 90:10 to 10:90.

[00064] According to another Aspect ("Aspect 61") further to any one of Aspects 50 to 59, where the inorganic particles having a spherical shape comprises a blend of 5 micron spherical particles and 10 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00065] According to another Aspect ("Aspect 62") further to any one of Aspects 50 to 59, the inorganic particles having a spherical shape comprises a blend of 5 micron spherical particles and 20 micron spherical particles, and where the blend is from 10:90 to 90:10.

[00066] According to another Aspect ("Aspect 63") further to any one of Aspects 50 to 62, including a dynamic binding capacity (DBC) of at least 35 mg/ml at a residence time of 20 seconds.

[00067] According to another Aspect ("Aspect 64") further to any one of Aspects 50 to 63, where a cycling durability of at least 100 cycles and an operating pressure less than 0.3 MPa.

[00068] According to another Aspect ("Aspect 65") further to any one of Aspects 50 to 64, where at least one upper intermediate material is positioned on a first side of the stacked membrane assembly and a lower intermediate material is positioned on a second side of the stacked membrane assembly, where the second side opposes the first side.

[00069] According to another Aspect ("Aspect 66") further to Aspect 65, where the first intermediate material and the second intermediate material are selected from a porous fluoropolymer film, a porous non-fluoropolymer film, a porous non-woven material and a porous woven material.

[00070] According to another Aspect ("Aspect 67") further to Aspect 66, where at least one of the first intermediate material and the second intermediate material is a polypropylene non-woven material.

[00071] In one Aspect ("Aspect 68") a manifold includes at least two of the affinity chromatography devices of any one of Aspects 50 to 67 arranged in a parallel configuration.

[00072] According to another Aspect ("Aspect 69") further to Aspect 68, where the manifold is contained within a housing.

[00073] In one Aspect ("Aspect 70") a device includes a first manifold and a second manifold in a parallel configuration and where each of the first manifold and the second manifold includes at least two of the affinity chromatography devices of any one of Aspects 50 to 69.

[00074] In another Aspect ("Aspect 71") further to Aspect 70, where the first manifold and the second manifold are enclosed within a housing.

[00075] In one Aspect ("Aspect 72") an article includes a diagnostic device that includes a fibrillated polymer membrane positioned between the fluid inlet and fluid outlet and containing therein inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns, where a particle size distribution has a D90/D10 less than or equal to 3, and where at least one of the fibrillated polymer membrane and the inorganic particles has covalently bonded thereto a ligand that reversibly binds to a targeted substance within a biological sample.

[00076] According to another Aspect ("Aspect 73") further to Aspect 72, including a fluid inlet and a fluid outlet fluidly connected to the fluid inlet.

[00077] According to another Aspect ("Aspect 74") further to Aspect 72 or Aspect 73, including a housing member encompassing the fluid inlet, the fluid outlet and the fibrillated polymer membrane

BRIEF DESCRIPTION OF THE DRAWINGS

[00078] 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.

[00079] FIG. 1 is an exploded view of a chromatography device containing a wound membrane assembly including a fibrillated heat treated polymer membrane containing therein spherical inorganic polymer particles according to at least one embodiment;

[00080] FIG. 2 is a cross section of a chromatography device depicting the outer flow channel and inner flow channel according to at least one embodiment in accordance with at least one embodiment;

[00081] FIG. 3 is a schematic cross-section of another spiral wound, normal flow chromatography device in accordance with at least one embodiment;

[00082] FIG. 4 is an exploded view of a chromatography device containing a stacked membrane assembly including a fibrillated heat treated polymer membrane containing therein spherical inorganic particles in accordance with at least one embodiment;

[00083] FIG. 5 is a graphical illustration depicting the relationship between dynamic binding capacity (DBC) and liquid permeability from the affinity chromatography device described in Example 1 in accordance with at least one embodiment;

[00084] FIG. 6 is a graphical illustration depicting the relationship between dynamic binding capacity (DBC) and liquid permeability from the affinity chromatography device described in Example 2 in accordance with at least one embodiment;

[00085] FIG. 7 is a schematic illustration of a front view of a manifold containing two chromatography devices arranged in a parallel configuration;

[00086] FIG. 8 is a schematic illustration of a top view of the manifold of FIG. 7;

[00087] FIG. 9 is a schematic illustration of a perspective view of a manifold containing four chromatography devices arranged in a parallel configuration;

[00088] FIG. 10 is a schematic illustration of a top view of the manifold of FIG. 9.

[00089] FIG. 11 is a schematic illustration of a front view of two manifolds arranged in a parallel configuration; and

[00090] FIG. 12 is a schematic illustration of a top view of the two manifolds of FIG. 11.

DETAILED DESCRIPTION

[00091] Persons skilled in the art will readily appreciate that various aspects of the present disclosure can be realized by any number of methods and apparatus configured to perform the intended functions. It should also be noted that the accompanying figures referred to herein are not necessarily drawn to scale, but may be exaggerated to illustrate various aspects of the present disclosure, and in that regard, the figures should not be construed as limiting. It is to be understood that, as used herein, the term “on” is meant to denote an element, such as a polymer membrane, is directly on another element or intervening elements may also be present. It is to be appreciated that the phrases “spherical particles”, “spherical inorganic particles”, and “inorganic particles having a spherical shape” may be interchangeably herein. Additionally, the term “spiral wound membrane assembly” is meant to include both the fibrillated heat treated polymer membrane alone and the fibrillated heat treated polymer membrane with the intermediate material. Further, the term “stacked membrane assembly” is meant to include both the fibrillated heat treated polymer membrane alone and the fibrillated heat treated polymer membrane with one or more intermediate material(s). The terms “fibrillated heat treated polymer membrane” and “heat treated fibrillated polymer membrane” may be used interchangeably herein. In addition, the terms “affinity chromatography device” and “chromatography device” may be used interchangeably herein.

[00092] The present disclosure is directed to affinity chromatography devices that separate a targeted molecule from an aqueous mixture containing the targeted molecule. The targeted molecule includes, but is not limited to, proteins, antibodies, viral vectors, and combinations thereof. The chromatography device includes a fibrillated heat treated polymer membrane that contains inorganic particles having a spherical shape and a particle size distribution that has a D90/D10 less than or equal to 3. In some embodiments, a blend or combination of various sizes of the spherical inorganic particles is utilized. A nominal particle size of the spherical inorganic particles is from about 5 microns to about 20 microns. An affinity ligand may be bonded to the spherical inorganic particles and/or to the fibrillated heat treated polymer membrane. The heat treatment of the polymer membrane results in a

significant and beneficial increase to device liquid permeability, subsequently enabling the desired shorter residence times while maintaining acceptable device pressures. The benefit of higher permeability from the heat treatment comes with a proportionately small decrement to device binding capacity (DBC). The chromatography devices have a dynamic binding capacity greater than 35 mg/ml at 10% breakthrough at a residence time of 20 seconds. In addition, the affinity chromatography devices have a cycling durability of at least 100 cycles without exceeding an operating pressure of 0.3 MPa. It is to be appreciated that the term “about” as used herein denotes $\pm 10\%$ of the designated unit of measure.

[00093] Looking at FIGS. 1 and 2, a wound chromatography device 100 is depicted. In forming the chromatography device 100, at least one fibrillated heat treated polymer membrane containing therein spherical inorganic particles is wrapped around a cylindrical core 150. Fibrillated, as used herein, refers to the inclusion of fibrils in a polymer membrane, such as, for example, a membrane having a microstructure characterized by nodes interconnected by fibrils where voids are the spaces between the nodes and fibrils. “Heat treatment”, as used herein, is meant to describe the end product of a heat treating step whereby the fibrillated polymer membrane is subjected to a temperature above its melting point for a designated period of time. In some embodiments, at least one inner intermediate material 200 may be circumferentially positioned against (*e.g.*, wound around) the core 150 to a desired width or a pre-designated amount. A fibrillated heat treated polymer membrane containing spherical inorganic particles therein 210 is then wound around the core 150 over the inner intermediate material 200 to a desired width or a pre-designated amount, and an outer layer of at least one outer intermediate material 220 is circumferentially positioned on (*e.g.*, wound around) the fibrillated heat treated polymer membrane 210 to a desired width or a pre-designated amount. Herein, the combination of the inner intermediate material 200, the fibrillated heat treated polymer membrane 210, and the outer intermediate material 220 will be referred to as the “wound membrane assembly”. The “wound membrane assembly”, in some embodiments, may include a fibrillated heat treated polymer membrane and an inner intermediate material(s), a fibrillated heat treated polymer membrane and an outer intermediate material(s), as well as any combination of polymers and/or polymer and intermediate material wrapped around a core. The cylindrical core 150 may have a hollow or solid interior. In either instance, the core 150 contains a solid outer wall so

that an aqueous mixture flowing through the chromatography device 100 flows within an inner flow channel formed of the inner intermediate material(s), which is discussed in detail below. The use of a hollow core 150 reduces the amount of material used to form the core 150, reduces the weight of the device 100, and reduces manufacturing costs.

[00094] The membrane assembly 110 and central core 150 may be positioned within a housing member 50. In some embodiments, the housing member 50 is cylindrical. In the embodiment depicted in FIGS. 1 and 2, the outer intermediate material(s) 220 forms an outer flow channel 130 and the inner intermediate material(s) 200 form an inner flow channel 140. It is to be appreciated that the intermediate material(s) 200, 220 in the embodiments described herein may be different or they may be the same. Additionally, two or more intermediate materials may be used to form one or both of the outer flow channel 130 and the inner flow channel 140. In use, an aqueous mixture flows into the inlet 80 positioned within inlet cap 60 where the mixture flows over the distributor cap 65 and is directed towards the outer flow channel formed by the outer intermediate material(s) 220. The distributor cap 65 directs the aqueous mixture 90 degrees from the feed direction towards the outer flow channel 130 (*i.e.*, intermediate material(s) 220). This redirection promotes a more uniform flow of the aqueous mixture into the outer flow channel 130. The outer intermediate layers forming 220 forming the outer flow channel 130 is located between the housing member 50 and the wound polymer membrane 210. The distributor cap 65 may be a polyolefin or coated with a polyolefin. It is to be appreciated that the aqueous mixture flows along an outer channel gap 165 and connects with the outer flow channel 130.

[00095] The aqueous mixture flows through the outer flow channel 130 (*i.e.*, outer intermediate material(s) 220) across the wound polymer membrane 210 in a normal direction (*e.g.*, a normal flow). As the aqueous mixture is passed in a normal flow from the outer flow channel 130 (*i.e.*, outer intermediate material(s) 220) and across the wound polymer membrane 210, the affinity ligand reversibly binds to the targeted protein, thereby effectively removing it from the aqueous mixture. The aqueous mixture then enters the inner flow channel 140 (*i.e.*, inner intermediate material(s) 200) located between the solid outer wall of the central core 150 and the wound polymer membrane 210

[00096] The aqueous mixture then is redirected at the bottom of the inner flow channel 140 by an outlet cap 75. The aqueous mixture then flows out of the chromatography device 100 through outlet 85 located within the outlet cap 75. It is to be appreciated that the diameter and/or height of the central core 150 (and/or the width and/or height of the fibrillated heat treated polymer membrane and/or intermediate material(s)) can be adjusted to achieve a larger volume without negatively impacting performance of the device. Additionally, the targeted protein may be removed from the affinity ligand, for example, by passing a fluid that has a lower pH through the chromatography device, as is known by those of ordinary skill in the art.

[00097] The intermediate material(s) 200, 220 is not particularly limiting so long as the aqueous mixture is able to flow therethrough. Some non-limiting examples of suitable intermediate materials include, but are not limited to, a porous fluoropolymer film or a porous non-fluoropolymer film (*e.g.*, a porous polypropylene or other porous polyolefin film), a porous non-woven material, or a porous woven material. In some embodiments, the wound membrane assembly includes an integrated inlet end cap 60 at one end of the core 150 and an integrated outlet end cap 75 at an opposing end of the core 150 to form an integrated, reusable chromatography device.

[00098] The total number of fibrillated polymer membrane layers present in the wound membrane assembly is not particularly limited, and depends on the desired end use and/or desired mass transit flow within the membrane assembly. The wound membrane assembly may include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 (or more) total polymer membrane layers. It is to be appreciated that hundreds or even thousands of polymer membrane layers may be present in the stacked membrane assembly. In addition, the fibrillated polymer membrane present in the wound membrane assembly may have a single layer thickness from about 1 micron to about 10,000 microns, from about 100 microns to about 5,000 microns, from about 500 microns to about 3,000 microns, or from about 650 microns to about 1,000 microns. As used herein, the term "thickness" is the direction of the fibrillated polymer membrane normal to the length area of the fibrillated polymer membrane.

[00099] The function of the chromatography device 300 depicted in FIG. 3 is substantially similar to the chromatography device 100 depicted in FIG. 2. For instance, an aqueous mixture is introduced into the chromatography device 300 via

the inlet 80 positioned within inlet cap 60 in the direction of arrow 62. The aqueous mixture is directed from the feed direction by the distributor cap 65. For ease of description, the aqueous mixture may flow in the direction depicted by arrow 55 towards the outer flow channel 130 (which may be formed of outer intermediate material(s) 220). The aqueous mixture flows along an outer channel gap 165 and connects with the inner flow channel 140 where it flows in the direction of arrow 30. The aqueous mixture flows across the wound polymer membrane 210 in a normal direction (*e.g.*, a normal flow) as shown by arrow 70 from the outer flow channel 130 to the outer flow channel 140. As the aqueous mixture passes through the wound polymer membrane 210, the affinity ligand reversibly binds to the targeted molecule. It is to be appreciated that the inner flow channel 140 may be formed of inner intermediate material(s) 200.

[000100] The aqueous mixture free of the targeted molecule flows down the outer flow channel 140 in the direction depicted by arrow 40. The aqueous mixture is redirected at the bottom of the inner flow channel 140 towards the central portion of the chromatography device 300 as depicted by arrow 52. The aqueous mixture free of the targeted molecule flows out of the chromatography device 300 through outlet 85 positioned within the outlet end cap 75 in the direction of arrow 45.

[000101] In other embodiments, such as is depicted generally in FIG. 4, the chromatography device 200 contains a fibrillated heat treated polymer membrane configured as individual discs 240 stacked upon each other to form a stacked membrane assembly 220. The fibrillated heat treated polymer membrane 240 may be positioned in a stacked configuration by simply laying the fibrillated heat treated polymer membrane discs 240 on top of each other. Alternatively, the fibrillated heat treated polymer membrane discs 240 may be stacked and subsequently laminated together with heat and/or pressure or other conventional methods. It is to be appreciated that the stacked membrane assembly 240 described herein is with respect to fibrillated heat treated polymer membrane discs for ease of explanation. The fibrillated heat treated polymer membrane formed in their geometric shape(s) and/or non-geometric shape(s) are considered to be within the purview of the disclosure.

[000102] The chromatography device 200 includes at least one upper intermediate material(s) 260 positioned at the top of the stacked membrane assembly and at least one lower intermediate material(s) 280 positioned at the

bottom of the stacked membrane assembly 220. The upper and lower intermediate material(s) 260, 280, respectively, may be the same or different. Similar to the wound membrane assembly discussed above, the intermediate material(s) 260, 280 used to form the stacked membrane assembly 220 is not particularly limiting so long as the aqueous mixture is able to flow therethrough. Non-limiting examples of suitable intermediate materials include, but are not limited to, a porous fluoropolymer film or a porous non-fluoropolymer film (*e.g.*, a porous polypropylene or other porous polyolefin film), a porous non-woven material, or a porous woven material.

[000103] The stacked membrane assembly 220 may be disposed within a housing 250 having an inlet cap 265 and an outlet end cap 275 disposed at opposite ends of the housing 250. In some embodiments, the housing 250 is cylindrical, although any geometry that is capable of housing the stacked membrane assembly and achieving a desired dynamic binding capacity is considered to be within the purview of this disclosure. In some embodiments, the intermediate material(s) 260, 280, the housing 250, the inlet cap 265, and the outlet cap 275 may be formed of a thermoplastic polymer such as polypropylene, polyethylene, or other polyolefins. Alternatively, one or both of the intermediate material(s) 260, 280 may be formed of an inorganic or metallic material, so long as the porous intermediate material(s) 260, 280 do not hinder the operation of the chromatography device.

[000104] The fibrillated heat treated polymer membranes 240 in the stacked membrane assembly 220 may be adhered to the housing 250 at the inner walls of the housing 250 via any conventional process (*e.g.*, melt sealing or use of a sealant) that prevents flow between the periphery of the fibrillated heat treated polymer membranes 240 and the housing 250. The inlet cap 265 and the outlet cap 275 may be sealed to the housing 250 by a similar or identical process. The inlet cap and the outlet cap 265, 275 includes an inlet 280 and an outlet 285, respectively, to permit the flow of an aqueous mixture through the affinity chromatography device 200. Specifically, the inlet cap 265 permits fluid flow of the aqueous mixture into the housing 250 and the outlet cap 285 permits fluid flow of the aqueous mixture out of the housing 250. In use, the aqueous mixture flows sequentially through the intermediate material(s) 260, through the fibrillated heat treated polymer membranes 240 forming the stacked membrane assembly 220, and through the intermediate material(s) 280. As the aqueous mixture is passed through the chromatography device 200, the affinity ligand reversibly binds to the targeted molecule, thereby

effectively removing it from the aqueous mixture. The targeted molecule may be removed from the affinity ligand, for example, by passing a fluid that has a lower pH through the device, as is known by those of skill in the art.

[000105] The total number of fibrillated heat treated polymer membranes present in the stacked membrane assembly is not particularly limited, and depends on the desired end use and/or desired mass transit flow within the membrane assembly. The stacked membrane assembly may include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 (or more) total polymer membranes. It is to be appreciated that hundreds or even thousands of polymer membranes may be present in the stacked membrane assembly. In addition, the fibrillated heat treated polymer membranes present in the stacked membrane assembly may have a thickness from about 1 micron to about 10,000 microns, from about 100 microns to about 5,000 microns, from about 500 microns to about 3,000 microns, or from about 650 microns to about 1,000 microns. As used herein, the term “thickness” is the direction of the fibrillated heat treated polymer membrane normal to the length area of the fibrillated heat treated polymer membrane.

[000106] The fibrillated heat treated polymer membranes in both the wound membrane assembly and the stacked membrane assembly contain spherical inorganic particles, or particles having a spherical configuration. As used herein, the term “spherical” is meant to denote that the inorganic particle has a round or nearly round shape where the distance from the center of the inorganic particle to the outer edge of the particle at any point is the same or nearly the same distance. In some embodiments, the spherical inorganic particles have a particle size distribution that has a D90/D10 less than or equal to 3, less than or equal to 2.5, less than or equal to 2, less than or equal to 1.5, or less than or equal to 1. The spherical inorganic particles have a nominal particle size that may be about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations and blends thereof. In some embodiments, the spherical inorganic particles are polydisperse.

[000107] In some embodiments, the fibrillated heat treated polymer membrane includes more than one nominal particle size and/or more than one type of spherical inorganic particle within the fibrillated heat treated polymer membrane. The fibrillated heat treated polymer membrane may contain from about 10 mass% fibrillated heat treated polymer membrane to about 90 mass% spherical inorganic particles, from about 15 mass% fibrillated heat treated polymer membrane to about

85 mass% spherical inorganic particles, from about 20 mass% fibrillated heat treated polymer membrane to about 80 mass% spherical inorganic particles, from about 30 mass% fibrillated heat treated polymer membrane to about 70 mass% spherical inorganic particles, from about 35 mass% fibrillated heat treated polymer membrane to about 65 mass% spherical inorganic particles, from about 40 mass% fibrillated heat treated polymer membrane to about 60 mass % spherical inorganic particles, from about 45 mass% fibrillated heat treated polymer membrane to about 55 mass% spherical inorganic particles, or from about 50 mass% fibrillated heat treated polymer membrane to about 50 mass% spherical inorganic particles. Non-limiting examples of suitable inorganic particles include silica, zeolites, hydroxyapatite, metal oxides, and combinations thereof. Additionally, the inorganic particles may be either solid or porous. Additionally, the affinity chromatography devices described herein have a hydraulic permeability from about 200 ($\times 10^{-12} \text{ cm}^2$) to about 700 ($\times 10^{-12} \text{ cm}^2$), from about 250 ($\times 10^{-12} \text{ cm}^2$) to about 700 ($\times 10^{-12} \text{ cm}^2$), from about 300 ($\times 10^{-12} \text{ cm}^2$) to about 650 ($\times 10^{-12} \text{ cm}^2$), from about 350 ($\times 10^{-12} \text{ cm}^2$) to about 600 ($\times 10^{-12} \text{ cm}^2$), from about 400 ($\times 10^{-12} \text{ cm}^2$) to about 600 ($\times 10^{-12} \text{ cm}^2$), from about 400 ($\times 10^{-12} \text{ cm}^2$) to about 550 ($\times 10^{-12} \text{ cm}^2$), from about 450 ($\times 10^{-12} \text{ cm}^2$) to about 550 ($\times 10^{-12} \text{ cm}^2$), from about 300 ($\times 10^{-12} \text{ cm}^2$) to about 500 ($\times 10^{-12} \text{ cm}^2$), from about 350 ($\times 10^{-12} \text{ cm}^2$) to about 500 ($\times 10^{-12} \text{ cm}^2$), or from about 400 ($\times 10^{-12} \text{ cm}^2$) to about 500 ($\times 10^{-12} \text{ cm}^2$).

[000108] In at least one embodiment, the fibrillated heat treated polymer membrane contains a blend of spherical inorganic particles having different nominal particle sizes. For example, the fibrillated heat treated polymer membrane may include a 90:10 mixture of a first nominal particle size (*e.g.*, 5 microns) and a second nominal particle size (*e.g.*, 20 microns) of the same or different spherical inorganic particle. The blend of spherical inorganic particles within the fibrillated heat treated polymer membrane may be any blend, such as, for example, a blend from 10:90 to 90:10, a blend from 30:70 to 70:30, a blend from 60:40 to 40:60, a blend from 25:75 to 75:25, a blend from 20:80 to 80:20, or a 50:50 blend. In one embodiment, the inorganic particles having a spherical shape includes 10 micron spherical particles and 20 micron spherical particles in a blend from 10:90 to 90:10. In another embodiment, the inorganic particles having a spherical shape includes 5 micron spherical particles and 10 micron spherical particles in a blend from 10:90 to 90:10. In a further embodiment, the inorganic particles having a spherical shape includes 5

micron spherical particles and 20 micron spherical particles in a blend from 10:90 to 90:10.

[000109] In some embodiments, the affinity ligand is covalently bonded to the spherical inorganic particles. In another embodiment, the affinity ligand is covalently bonded to the fibrillated heat treated polymer membrane. In a further embodiment, the affinity ligand may be bound to both the polymer membrane and the spherical inorganic particle(s). The affinity ligand may be a protein, antibody, or polysaccharide that reversibly binds to the targeted molecule. In one embodiment, the affinity ligand is a protein that reversibly binds, for example, to an Fc region of an antibody, an antibody fragment, an Fc fusion protein, or an antibody/drug conjugate. In another embodiment, the affinity ligand is an antibody, Protein L, or a polysaccharide that reversibly binds to a protein or a protein fragment to which it is specific. Exemplary affinity ligands for use in the affinity chromatography device include, but are not limited to, Protein A, Protein G, Protein L, human Fc receptor protein, antibodies that specifically bind to other proteins, and heparin. The affinity ligand may be native, recombinant, or synthetic. In yet another embodiment, the affinity ligand is a metal affinity ligand that reversibly binds to His-Tagged Proteins. In yet another embodiment, the affinity ligand may be an antibody or a polysaccharide that reversibly binds to a viral vector for which it is specific.

[000110] In at least one embodiment, the fibrillated heat treated fluoropolymer membrane is an expanded polytetrafluoroethylene (ePTFE) membrane. Expanded polytetrafluoroethylene (ePTFE) membranes prepared in accordance with the methods described in U.S. Patent No. 7,306,729 to Bacino *et al.*, U.S. Patent No. 3,953,566 to Gore, U.S. Patent No. 5,476,589 to Bacino, or U.S. Patent No. 5,183,545 to Branca *et al.* may be used herein. Further, the fibrillated heat treated fluoropolymer membrane may be rendered hydrophilic (*e.g.*, water-wettable) using known methods in the art, such as, but not limited to, the method disclosed in U.S. Patent No. 4,113,912 to Okita, *et al.* A coating that effectively binds to a ligand, such as described in U.S. Patent No. 5,897,955 to Drumheller, U.S. Patent No. 5,914,182 to Drumheller, or U.S. Patent No. 8,591,932 to Drumheller may be applied to the fibrillated heat treated polymer membrane.

[000111] The heat treated fibrillated fluoropolymer membrane may also include a polymer material including a functional tetrafluoroethylene (TFE) copolymer membrane where the functional TFE copolymer material includes a functional

copolymer of TFE and PSVE (perfluorosulfonyl vinyl ether), or TFE with another suitable functional monomer, such as, but not limited to, vinylidene fluoride (VDF), vinyl acetate, or vinyl alcohol.

[000112] It is to be understood that throughout the application, the term “PTFE” is utilized herein for convenience and is meant to include not only polytetrafluoroethylene, but also expanded PTFE, expanded modified PTFE, and expanded copolymers of PTFE, such as described in U.S. Patent No. 5,708,044 to Branca, U.S. Patent No. 6,541,589 to Baillie, U.S. Patent No. 7,531,611 to Sabol et al., U.S. Patent No. 8,637,144 to Ford, and U.S. Patent No. 9,139,669 to Xu, *et al.*

[000113] The intermediate material(s) may be a fluoropolymer film or a non-fluoropolymer film (*e.g.*, polyethylene, expanded polyethylene, or other polyolefin film). Additionally, the intermediate film may be porous. In some embodiments, the intermediate film is a thermoplastic or thermoset polymer film.

[000114] Advantageously, the chromatography device may be used multiple times. Additionally, the chromatography device may be cleaned with a cleaning solution (*e.g.* sodium hydroxide, phosphoric acid, citric acid, ethanol, and the like) after each separation process or after multiple separation processes and reused.

[000115] The affinity chromatography devices described herein have a dynamic binding capacity (DBC) greater than 35 mg/ml at a residence time of 20 seconds. In addition, the affinity chromatography devices have a cycling durability of at least 100 cycles without exceeding an operating pressure of 0.3 MPa. In addition, the chromatography devices may be used multiple times without losing substantial dynamic binding capacity. Specifically, the chromatography devices may be cleaned with a cleaning solution (*e.g.* sodium hydroxide) after each separation process and reused. Although embodiments of the wound membrane assembly 110 and stacked membrane assembly 220 are described herein, it is to be appreciated that any number of fibrillated heat treated polymer membranes as well as any and all combinations of types of fibrillated heat treated polymer membranes, types of spherical inorganic particles, sizes of spherical inorganic particles, and orientations of the fibrillated heat treated polymer membrane(s) within the membrane assemblies 110, 220 are within the scope of this disclosure. Also, some or all of the fibrillated heat treated polymer membranes may vary in composition, thickness, permeability, etc. from each other.

[000116] The chromatography devices described herein and components thereof can be fabricated using various processes. In some embodiments, injection molding may be used to fabricate the chromatography components provided herein. Other suitable processes can include, but are not limited to, extrusion, compression molding, solvent casting, and combinations thereof. Embodiments employing two fibrillated heat treated polymer membranes that are co-expanded to produce a composite membrane assembly is also considered to be within the purview of the disclosure. Such a composite membrane assembly may contain two (or more) layers of fibrillated heat treated polymer membranes that may be co-extruded or integrated together.

[000117] In some embodiments, the affinity chromatography devices described herein utilized in a dual manifold 750 with affinity chromatography devices 700, 701 arranged in a parallel configuration, such as is generally depicted in FIG. 7. It is to be appreciated that the chromatography devices depicted in FIGS. 7-12 contain a stacked membrane assembly, a wound membrane assembly, or a combination of stacked and wound membrane assemblies. As shown, an aqueous mixture flows through a main tube 720 to a distribution element 740. The distribution element 740 is not particularly limiting so long as it divides the flow of the aqueous mixture coming from the main tube 720 into at least two inlet tubes 760, 761. The split aqueous mixture in inlet tubes 760, 761 flows into chromatography devices 700, 701, where the targeted molecule is captured. An aqueous solution (*i.e.*, the aqueous mixture minus the targeted molecule captured by the affinity ligand) flows out of the chromatography devices 700, 701 through outlet tubes 780, 781, respectively. The aqueous solution in outlet tubes 780, 781 are combined in distribution element 790 and reconstituted into a single aqueous solution that flows into tube 795. A top view of the dual manifold 750 containing the parallel affinity chromatography devices 700, 701 is depicted in FIG. 8. In some embodiments, the manifold 750 is positioned within a housing 720.

[000118] It is to be appreciated that two chromatography devices 700, 701 are shown in FIGS. 7 and 8 for illustration only and a plurality (*i.e.* three or more) chromatography devices described herein may be utilized in a manifold in a parallel configuration so long as there is a similar distribution of flow and resistance across the chromatography devices. This similarity across the affinity chromatography devices allows for scalability of device volume and performance. The affinity

chromatography devices utilized in the manifold (e.g., dual, quad, etc.) may be the same or different from each other. In addition, the affinity chromatography devices may be dropped into a parallel configuration system without any changes or additions to the manifold 750.

[000119] An example of a manifold containing a plurality of chromatography devices as described herein is a quad manifold depicted in FIG. 9. A top view of this quad manifold 950 is shown in FIG. 10. Similar to the dual manifold depicted in FIGS. 7 and 8, an aqueous mixture flows into a distribution element 940. The distribution element 940 divides the flow of an aqueous mixture into inlet tubes 960, 961, 962, and 963. It is to be noted that 962 and 963 are hidden behind 961 and 962, respectively in FIG. 9. Likewise, 903 and 904 are hidden behind 901 and 902, respectively. The split aqueous mixture in inlet tubes 960, 961, 962, and 963 flows into the four chromatography devices 900, 901, 902, 903, respectively, where the targeted molecule is captured. An aqueous solution flows out of each of the chromatography devices 900, 901, 902, and 903 (902 and 903 not illustrated) through outlet tubes 980, 981, 982, and 983, respectively (982 and 983 not illustrated). The aqueous solution in outlet tubes 980, 981 (982 and 983 are hidden behind 980 and 981, respectively and are therefore not illustrated) are combined in a distribution element 990 and reconstituted into a single aqueous solution. In some embodiments, the quad manifold 950 may be positioned within a housing 920.

[000120] FIG. 11 depicts a device 1000 containing two manifolds 1150, 1151 in a parallel configuration. A top view of the device 1000 is depicted in FIG. 12. The manifolds 1150, 1151 each contain four affinity chromatography devices, of which, two affinity chromatography devices 1100, 1101 and 1102, 1103 of each manifold 1150, 1151 is depicted in FIG. 11. The ability to utilize at least two manifolds in parallel advantageously allows for an increase in volume capacity while utilizing the chromatography devices described herein. In other words, the device 1000 eliminates the need to move to a chromatography device that is larger in volume. In addition, placing the manifolds in parallel, such as is depicted in FIG. 11, reduces concerns of over pressurizing the device 1000.

[000121] In use, an aqueous mixture flows into a distribution element 1140. The distribution element 1140 divides the flow of an aqueous mixture into two distribution tubes 1141, 1142. The split aqueous mixture in distribution tubes 1141, 1142 is then further divided by distribution elements 1145, 1146 into inlet tubes. In the

embodiment depicted in FIG. 11, of the four inlet tubes of manifold 1150, inlet tubes 1160, 1161 are depicted. Similarly, of the four inlet tubes of manifold 1151, inlet tubes 1162, 1163 are depicted. It is to be appreciated that the remaining inlet tubes 1164, 1165, 1166, 1167 are hidden behind inlet tubes 1160, 1161, 1162, 1163 in FIG. 11. The aqueous mixture in the inlet tubes of the first and second manifold 1150, 1151 flow into the affinity chromatography devices. It is to be appreciated that in manifold 1150, affinity chromatography devices 1100, 1101 are depicted and in manifold 1151, affinity chromatography devices 1102, 1103 are depicted. The remaining chromatography devices 1104, 1105, 1106, 1107 are hidden behind affinity chromatography devices 1100, 1101, 1102, 1103 in FIG. 11. The targeted molecule is captured in the affinity chromatography devices.

[000122] An aqueous solution flows out of each of the chromatography devices through outlet tubes. In FIG. 11, outlet tubes 1180, 1181 of manifold 1150 and outlet tubes 1182, 1183 of manifold 1151 are depicted. It is to be appreciated that the remaining outlet tubes are hidden behind outlet tubes 1180, 1181, 1182, 1183 in FIG. 11. The aqueous solution in the outlet tubes are combined in distribution element 1170 and distribution element 1171. The combined solution then flows from the distribution elements 1170, 1170 into collection tubes 1110, 1111. The aqueous solution in collection tubes 1110, 1111 are combined in distribution element 1190 and reconstituted into a single aqueous solution. The first manifold 1150 and the second manifold 1151 may be enclosed within a housing 1120.

[000123] In some embodiments, the present disclosure is directed to a diagnostic device that removes a targeted substance from a biological sample. The device includes a heat treated fibrillated polymer membrane that contains therein inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns. The particle size distribution has a D90/D10 less than or equal to 3 and at least one of the heat treated fibrillated polymer membrane and the inorganic particles has covalently bonded thereto a ligand that reversibly binds to the targeted substance within a biological sample. The device may further include a fluid inlet and a fluid outlet fluidly connected to the fluid inlet. Additionally, the device may include a housing member encompassing the fluid inlet, the fluid outlet and the fibrillated polymer membrane.

[000124] Persons skilled in the art will readily appreciate that various aspects of the present disclosure can be realized by any number of methods and apparatus

configured to perform the intended functions. It should also be noted that the accompanying figures referred to herein are not necessarily drawn to scale, but may be exaggerated to illustrate various aspects of the present disclosure, and in that regard, the figures should not be construed as limiting.

TEST METHODS

[000125] It should be understood that although certain methods and equipment are described below, other methods or equipment determined suitable by one of ordinary skill in the art may be alternatively utilized.

Method for determining air permeation of membranes

[000126] Membrane air permeation was measured using a Gurley Densometer (Gurley Precision Instruments, Troy, NY). Time to permeate a 100 cc volume of air across a 1" sample diameter was recorded.

Method for determining particle size and particle size distribution

[000127] Particle size and particle size distribution data were supplied by the manufacturer and were measured using the Coulter counter technique.

Method for determining PTFE melt temperature

[000128] Differential Scanning Calorimetry (Model Q2000, TA Instruments, New Castle, DE) was used to characterize PTFE melt temperature within the membrane. Heat flow was measured on 4 mm diameter membrane samples ramping at 10 degrees/min from -40C to 400C in a nitrogen atmosphere. Melt temperature was recorded as the maximum in the associated endothermic peak.

Method for Determining the Dynamic Binding Capacity at 10% Breakthrough

[000129] The chromatography device was inserted in an AKTA™ Pure (Cytiva, Marlborough, MA) liquid chromatography system's flow path and a single cycle consisting of the following protocol was performed. Table A sets forth the solutions utilized, Table B sets forth the protocol steps to determine the dynamic binding capacity at 10% breakthrough.

Table A

Solution	Description
A	50 mM Sodium Phosphate supplemented with 150 mM Sodium Chloride, pH~7.4
B	100 mM Citrate, pH~3.4
CIP	0.1 M NaOH
Feed	2.9-3.0 mg/ml polyclonal IgG (Lee Biosciences) dissolved in Solution A
Storage	20/80 v/v ethanol/water

Table B

Step	Solution	Volume of Solution Used (Number of Bed Volumes)	Bed Volume/Volumetric Flow Rate = Seconds Residence Time
1	A	6	20
2	Feed	Until Absorbance at 280 nm = 10% Breakthrough	20
4	A	8	20
5	B	6	20
6	A	6	20
7	CIP	15 min contact time at 1 CV/min	N/A
8	A	5	20
9	Storage	6	20

Method for determining liquid permeability

[000130] The liquid permeability of the chromatography devices was determined using Darcy's law. Individual devices were characterized for bed cross sectional area and bed length. Solution A was used as the liquid and was characterized for viscosity. The pressure drop across the column as a function of liquid flux was measured on an AKTA™ Pure liquid chromatography system.

EXAMPLES**Example 1**

[000131] Spherical silica particles of size and size distribution described in Table C were obtained. The porous particles in Table C were substantially the same with respect to chemical and physical characteristics such as chemical composition, particle shape, nominal particle porosity, nominal particle pore dimensions and nominal particle surface areas.

Table C

Nominal Porous Silica Particle Size (micron)	Nominal Porous Silica Pore Size (nm)	Measured Average Silica Particle Size (um)	Porous Silica Particle Size Distribution (D90/D10)
5	100	3.8	1.48
10	100	8.2	1.29
20	100	14.4	1.36

[000132] Porous polytetrafluoroethylene (PTFE) membranes having 15 mass percent PTFE and 85 mass percent porous silica particles of various particle size ratios from Table C were obtained. These ratios are shown in Table D.

Table D

Porous Membrane	Percent 20 micron nominal particle size	Percent 10 micron nominal particle size	Percent 5 micron nominal particle size
A	70	30	0
B	50	50	0
C	30	70	0
D	0	70	30

[000133] Adjacent sections of the porous membranes described in Table D were affixed to a frame and subjected to a heat treatment process of 370°C for 20 seconds, then removed and cooled to room temperature while still held in the frame. Table E lists the physical characteristics and air permeation values of the polymer membranes before and after the heat treatment process.

Table E

Porous Membrane	Pre-treatment membrane thickness (mm)	Post-treatment membrane thickness (mm)	Pre-treatment Bulk Density (g/cc)	Post-treatment Bulk Density (g/cc)	Pre-treatment Gurley Permeation (s)	Post-treatment Gurley Permeation (s)
A	0.661	0.694	0.466	0.457	15.7	8.7
B	0.664	0.687	0.45	0.437	17.4	10
C	0.647	0.687	0.442	0.437	16.9	9.2
D	0.597	0.653	0.44	0.436	30.3	18.3

[000134] Porous membranes A and D were characterized for PTFE melt temperature before and after the heat treatment. The results are shown in Table F.

Table F

Porous Membrane	Pre Treatment PTFE Melt Temperature (Degrees Celsius)	Post Treatment PTFE Melt Temperature (Degrees Celsius)
A	344.6	333.3
D	344.6	333.8

[000135] The porous membranes from Table E, both pre- and post- heat treatment, were used to manufacture affinity chromatography devices. A polypropylene flow distributor was affixed to one end of a polypropylene cylinder housing. A porous polypropylene intermediate material was placed in the housing. The desired number of PTFE membrane layers were stacked on the polypropylene intermediate material within the housing as shown in Table G. A second porous polypropylene intermediate material was placed on top of the PTFE membrane stack. A second polypropylene flow distributor was affixed to the end of the cylindrical housing opposite the first polypropylene flow distributor. The chromatography device was sealed via a heating process.

[000136] The intermediate devices were then treated in a manner to covalently bond Protein A to the stacked membranes. This manner is representative of that typical to those skilled in the art and is further delineated in U.S. Patent No. 10,525,376 to McManaway and in U.S. Patent No. 10,525,367 to McManaway, *et al.*

[000137] The affinity chromatography devices whose manufacture was described above were tested to evaluate their liquid permeability and twenty (20) second residence time dynamic binding capacities using the protocols described in the Test Methods set forth herein. The performance of each of these affinity chromatography devices is shown in Table G.

Table G

Device Designation	Porous Membrane Used	Bed Volume (mL)	Device Liquid Permeability ($\times 10^{-12} \text{ cm}^2$)	Device Dynamic Binding Capacity (mg/mL) at 20 Seconds Residence Time
A	8 layers of membrane A	0.995	331.0	41.8
B	8 layers of heat treated membrane A	1.006	586.3	37.3
C	8 layers of membrane B	1.003	316.0	45.9
D	8 layers of heat treated membrane B	1.035	496.5	43.0
E	8 layers of membrane C	0.986	325.3	48.4
F	8 layers of heat treated membrane C	1.024	485.5	45.1
G	9 layers of membrane D	1.014	215.6	55.6
H	8 layers of heat treated membrane D	1.000	322.4	50.6

[000138] The relative impact of the membrane heat treatment process to the liquid permeability and dynamic binding capacity shown in Table G are represented graphically in FIG. 5.

Example 2

[000139] Porous membranes of Type A from Table E, both pre- and post- heat treatment, were used to manufacture affinity chromatography devices. A polypropylene flow distributor was affixed to one end of a polypropylene cylinder

housing. A porous polypropylene intermediate material was placed in the housing. The desired number of PTFE membrane layers were stacked on the polypropylene intermediate material within the housing as shown in Table H. A second porous polypropylene intermediate material was placed on top of the PTFE membrane stack. A second polypropylene flow distributor was affixed to the end of the cylindrical housing opposite the first polypropylene flow distributor. The chromatography device was sealed via a heating process.

[000140] As described in Example 1, the intermediate devices were then treated in a manner to covalently bond Protein A to the stacked membranes and then tested to evaluate their liquid permeability and twenty (20) second residence time dynamic binding capacities. The performance of each of these affinity chromatography devices is shown in Table H.

Table H

Device Designation	Porous Membrane Used	Bed Volume (mL)	Device Liquid Permeability ($\times 10^{-12} \text{ cm}^2$)	Device Dynamic Binding Capacity (mg/mL) at 20 Seconds Residence Time
I	9 layers of membrane A	2.970	408.1	39.85
J	9 layers of membrane A	2.999	399.7	39.25
K	9 layers of heat treated membrane A	3.033	741	36.41
L	9 layers of heat treated membrane A	3.045	787.7	37.01

[000141] Devices I and J were manifolded together in a parallel configuration into Device M such as is depicted in FIGS. 7 and 8. Devices K and L were similarly manifolded in a parallel configuration into Device N.

[000142] The affinity chromatography devices M and N, whose manufacture was described above, were tested to evaluate their liquid permeability and twenty (20) second residence time dynamic binding capacities using the protocols described in the Test Methods set forth herein. The performance of each of these affinity chromatography devices is shown in Table I.

Table I

Manifolded Device Designation	Devices used	Manifolded Bed Volume (mL)	Device Liquid Permeability ($\times 10^{-12} \text{ cm}^2$)	Device Dynamic Binding Capacity (mg/mL) at 20 Seconds Residence Time
M	I, J	5.969	410.1	39.54
N	K, L	6.078	742.4	36.52

[000143] The relative impact of the membrane heat treatment process to the liquid permeability and dynamic binding capacity shown in Table H are represented graphically in FIG. 6 and can be compared to the data set forth in FIG. 5.

[000144] The invention of this application has been described above both generically and with regard to specific embodiments. It will be apparent to those skilled in the art that various modifications and variations can be made in the embodiments without departing from the scope of the disclosure. Thus, it is intended that the embodiments cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

WHAT IS CLAIMED IS:

1. An article comprising:
an affinity chromatography device including:
a fluid inlet;
a fluid outlet fluidly connected to the fluid inlet;
a fibrillated heat treated polymer membrane positioned between the fluid inlet and fluid outlet and containing therein inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns;
a housing encompassing the fluid inlet, the fluid outlet and the fibrillated heat treated polymer membrane,
wherein a particle size distribution has a D90/D10 less than or equal to 3, and
wherein at least one of the fibrillated heat treated polymer membrane and the inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted molecule.
2. The article of claim 1, wherein the targeted molecule is a protein, antibody, viral vector and combinations thereof.
3. The article of claim 1 or claim 2, comprising a hydraulic permeability from about 200 ($\times 10^{-12} \text{ cm}^2$) to about 700 ($\times 10^{-12} \text{ cm}^2$).
4. The article of any one of claims 1 to 3, wherein the inorganic particles having a spherical shape are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.
5. The article of any one of claims 1 to 4, wherein the fibrillated heat treated polymer membrane comprises an expanded polytetrafluoroethylene membrane, an expanded modified polytetrafluoroethylene membrane, an expanded tetrafluoroethylene copolymer membrane, or an expanded polyethylene membrane.
6. The article of any one of claims 1 to 5, wherein the fibrillated polymer membrane is an expanded polytetrafluoroethylene membrane.

7. The article of any one of claims 1 to 6, wherein the affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.
8. The article of any one of claims 1 to 7, wherein the inorganic particles comprise at least a first inorganic particle having a spherical shape and a first nominal particle size and a second inorganic particle having a spherical shape and a second nominal particle size, the first and second nominal particle sizes being different from each other.
9. The article of any one of claims 1 to 8, wherein the nominal particle size is selected from about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations thereof.
10. The article of any one of claims 1 to 9, wherein the inorganic particles having a spherical shape comprise a blend of 10 micron spherical particles and 20 micron spherical particles, and wherein the blend is from 10:90 to 90:10.
11. The article of any one of claims 1 to 9, wherein the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 10 micron spherical particles, and wherein the blend is from 10:90 to 90:10.
12. The article of any one of claims 1 to 9, wherein the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 20 micron spherical particles, and wherein the blend is from 10:90 to 90:10.
13. The article of any one of claims 1 to 12, comprising a dynamic binding capacity (DBC) of at least 35 mg/ml at a residence time of 20 seconds.
14. The article of any one of claims 1 to 13, comprising a cycling durability of at least 100 cycles at an operating pressure less than 0.3 MPa.
15. The article of any one of claims 1 to 14, wherein the fibrillated heat treated heat treated polymer membrane has a wound configuration.

16. The article of any one of claims 1 to 15, wherein the fibrillated polymer membrane has a stacked configuration.
17. The article of any one of claims 1 to 16, wherein the fibrillated polymer membrane has a wound configuration, a stacked configuration, and a combination thereof.
18. The article of any one of claims 1 to 17, wherein an inner intermediate material is circumferentially positioned on an outer surface of a core and wherein the fibrillated heat treated polymer membrane is circumferentially positioned on the inner intermediate material.
19. The article of any one of claims 1 to 18, comprising an outer intermediate material circumferentially positioned on the heat treated fibrillated polymer membrane.
20. The article of any one of claims 1 to 19, wherein the inner intermediate material and the outer intermediate material are selected from a porous fluoropolymer film, a porous non-fluoropolymer film, a porous non-woven material and a porous woven material.
21. The article of any one of claims 1 to 20, wherein at least one of the inner intermediate material and the outer intermediate material is a polypropylene non-woven material.
22. A manifold comprising at least two of the affinity chromatography devices of any one of claims 1 to 21 arranged in a parallel configuration.
23. The manifold of claim 22, wherein the manifold is contained within a housing.
24. Use of the article of any one of claims 1 to 21 to separate the targeted molecule from a fluid stream.

25. A device comprising a first manifold and a second manifold in a parallel configuration, wherein each of the first manifold and the second manifold includes at least two of the affinity chromatography devices of any one of claims 1 to 21.
26. The device of claim 25, wherein the first manifold and the second manifold are enclosed within a housing.
27. An article comprising:
a centrally located core;
a fibrillated heat treated polymer membrane containing therein spherical inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns wound around the core;
a housing surrounding both the core and the fibrillated heat treated polymer membrane,
a first end cap positioned at a first end of the housing, and
a second end cap positioned at a second end of the housing,
wherein a particle size distribution has a D90/D10 less than 3, and
wherein at least one of the fibrillated heat treated polymer membrane and the spherical inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted molecule.
28. The article of claim 27, wherein the targeted molecule is a protein, antibody, viral vector and combinations thereof.
29. The article of claim 27 or claim 28 comprising a hydraulic permeability from about 200 ($\times 10^{12} \text{ cm}^2$) to about 700 ($\times 10^{12} \text{ cm}^2$).
30. The article of any one of claims 27 to 29, wherein the inorganic particles having a spherical shape are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.
31. The article of any one of claims 27 to 30, wherein the fibrillated heat treated polymer membrane includes at least one of an expanded polytetrafluoroethylene

membrane, an expanded modified polytetrafluoroethylene membrane, an expanded tetrafluoroethylene copolymer membrane, or an expanded polyethylene membrane.

32. The article of any one of claims 27 to 31, wherein the fibrillated heat treated polymer membrane is a fibrillated heat treated expanded polytetrafluoroethylene membrane.

33. The article of any one of claims 27 to 32, wherein the affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.

34. The article of any one of claims 27 to 33, wherein the inorganic particles comprise at least a first inorganic particle having a spherical shape and a first nominal particle size and a second inorganic particle having a spherical shape and a second nominal particle size, the first and second nominal particle sizes being different from each other.

35. The article of any one of claims 27 to 33, wherein the nominal particle size is selected from about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations thereof.

36. The article of any one of claims 27 to 33, wherein the inorganic particles having a spherical shape comprise a blend of 10 micron spherical particles and 20 micron spherical particles, and wherein the blend is from 10:90 to 90:10.

37. The article of any one of claims 27 to 31, wherein the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 10 micron spherical particles, and wherein the blend is from 10:90 to 90:10.

38. The article of any one of claims 27 to 33, wherein the inorganic particles having a spherical shape comprise a blend of 5 micron spherical particles and 20 micron spherical particles, and wherein the blend is from 10:90 to 90:10.

39. The article of any one of claims 27 to 38, comprising a dynamic binding capacity (DBC) of at least 35 mg/ml at a residence time of 20 seconds.
40. The article of any one of claims 27 to 39, comprising a cycling durability of at least 100 cycles and an operating pressure less than 0.3 MPa.
41. The article of any one of claims 27 to 40, wherein an inner intermediate material is circumferentially positioned on an outer surface of a core and the fibrillated heat treated polymer membrane is circumferentially positioned on the inner intermediate material.
42. The article of any one of claims 27 to 41, comprising an outer intermediate material circumferentially positioned on the fibrillated heat treated polymer membrane.
43. The article of any one of claims 27 to 42, wherein the inner intermediate material and the outer intermediate material are selected from a porous fluoropolymer film, a porous non-fluoropolymer film, a porous non-woven material and a porous woven material.
44. The article of any one of claims 27 to 43, wherein at least one of the inner intermediate material and the outer intermediate material is a polypropylene non-woven material.
45. A manifold comprising at least two of the affinity chromatography devices of any one of claims 27 to 44 arranged in a parallel configuration.
46. The manifold of claim 45, wherein the manifold is enclosed within a housing.
47. A device comprising a first manifold and a second manifold in a parallel configuration, wherein each of the first manifold and the second manifold includes at least two of the affinity chromatography devices of any one of claims 27 to 44.

48. The device of claim 47, wherein the first manifold and the second manifold are enclosed within a housing.

49. Use of the article of any one of claims 27 to 44 to separate the targeted molecule from a fluid stream.

50. An article comprising:
an affinity chromatography device including:
a housing member;
an inlet to permit fluid flow into said housing;
an outlet to permit fluid flow out of said housing, and
a stacked membrane assembly disposed within said housing between the fluid inlet and fluid outlet, said stacked membrane assembly comprising:
two or more fibrillated heat treated polymer membranes in a stacked configuration, each said fibrillated polymer membrane containing therein inorganic particles having a spherical shape and a blend of nominal particle sizes, the nominal particle sizes being from about 5 microns to about 20 microns,
wherein a particle size distribution has a D90/D10 less than 3, and
wherein at least one of the fibrillated heat treated polymer membrane and the spherical inorganic particles has covalently bonded thereto an affinity ligand that reversibly binds to a targeted molecule.

51. The article of claim 50, wherein the targeted molecule is a protein, antibody, viral vector and combinations thereof.

52. The article of claim 50 or claim 51, comprising first and second flow distributors, said first flow distributor and said second flow distributor positioned at opposing ends of said housing.

53. The article of any one of claims 50 to 52, comprising a hydraulic permeability from about 200 ($\times 10^{-12} \text{ cm}^2$) to about 700 ($\times 10^{-12} \text{ cm}^2$).

54. The article of any one of claims 50 to 53 wherein the inorganic particles having a spherical shape are selected from silica, zeolites, hydroxyapatite, metal oxides and combinations thereof.
55. The article of any one of claims 50 to 54, wherein the fibrillated heat treated polymer membrane includes an expanded polytetrafluoroethylene membrane, an expanded modified polytetrafluoroethylene membrane, an expanded tetrafluoroethylene copolymer membrane, or an expanded polyethylene membrane.
56. The article of any one of claims 50 to 55, wherein the fibrillated heat treated polymer membrane is a fibrillated heat treated expanded polytetrafluoroethylene membrane.
57. The article of any one of claims 50 to 56, wherein the affinity ligand is selected from Protein A, Protein G, Protein L, human Fc receptor protein, antibodies, polysaccharides and combinations thereof.
58. The article of any one of claims 50 to 57, wherein the inorganic particles comprise at least a first inorganic particle having a spherical shape and a first nominal particle size and a second inorganic particle having a spherical shape and a second nominal particle size, the first and second nominal particle sizes being different from each other.
59. The article of any one of claims 50 to 58, wherein the nominal particle size is selected from about 5 microns, about 10 microns, about 15 microns, about 20 microns, and combinations thereof.
60. The article of any one of claims 50 to 59, wherein the inorganic particles having a spherical shape comprises a blend of 10 micron spherical particles and 20 micron spherical particles, and wherein the blend is from 90:10 to 10:90.
61. The article of any one of claims 50 to 59, wherein the inorganic particles having a spherical shape comprises a blend of 5 micron spherical particles and 10 micron spherical particles, and wherein the blend is from 10:90 to 90:10.

62. The article of any one of claims 50 to 59, wherein the inorganic particles having a spherical shape comprises a blend of 5 micron spherical particles and 20 micron spherical particles, and wherein the blend is from 10:90 to 90:10.
63. The article of any one of claims 50 to 62, comprising a dynamic binding capacity (DBC) of at least 35 mg/ml at a residence time of 20 seconds.
64. The article of any one of claims 50 to 63, comprising a cycling durability of at least 100 cycles an operating pressure less than 0.3 MPa.
65. The article of any one of claims 50 to 64, wherein at least one first intermediate material is positioned on a first side of the stacked membrane assembly and a second intermediate material is positioned on a second side of the stacked membrane assembly, the second side opposing the first side.
66. The article of claim 65, wherein the first intermediate material and the second intermediate material are selected from a porous fluoropolymer film, a porous non-fluoropolymer film, a porous non-woven material and a porous woven material.
67. The article of claim 67, wherein at least one of the first intermediate material and the second intermediate material is a polypropylene non-woven material.
68. A manifold comprising at least two of the affinity chromatography devices of any one of claims 50 to 67 arranged in a parallel configuration.
69. The manifold of claim 68, wherein the manifold is enclosed within a housing.
70. A device comprising a first manifold and a second manifold in a parallel configuration, wherein each of the first manifold and the second manifold includes at least two of the affinity chromatography devices of any one of claims 50 to 69.
71. The device of claim 70, wherein the first manifold and second manifold are enclosed within a housing.

72. An article comprising:
A diagnostic device including:
a fibrillated polymer membrane positioned between the fluid inlet and fluid outlet and containing therein inorganic particles having a spherical shape and a nominal particle size from about 5 microns to about 20 microns,
wherein a particle size distribution has a D90/D10 less than or equal to 3, and
wherein at least one of the fibrillated polymer membrane and the inorganic particles has covalently bonded thereto a ligand that reversibly binds to a targeted substance within a biological sample.
73. The article of claim 72, comprising a fluid inlet and a fluid outlet fluidly connected to the fluid inlet.
74. The article of claim 72 or claim 73, comprising a housing member encompassing the fluid inlet, the fluid outlet and the fibrillated polymer membrane.