

CHROMATOGRAPHY DEVICE AND METHOD OF USE

BACKGROUND OF THE INVENTION

[0001] RNA (e.g., lentivirus RNA) and DNA (e.g., plasmid DNA) purification typically requires the use of different chromatography devices and dilution of high salt into low conductivity buffer.

[0002] There is a need for improved chromatography devices and methods of purification.

[0003] The present invention provides for ameliorating at least some of the disadvantages of the prior art. These and other advantages of the present invention will be apparent from the description as set forth below.

BRIEF SUMMARY OF THE INVENTION

[0004] An aspect of the invention provides a chromatography device comprising (a) a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet; (b) a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising (i) a first porous filter element; and (ii) a second porous filter element in contact with the first porous filter element, the second porous filter element arranged downstream of the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one hydrophobic interaction (HIC) layer.

[0005] Another aspect of the invention provides a method of purification of nucleic acids, the method comprising passing a fluid comprising nucleic acid through a chromatography device comprising (a) a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet; (b) a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising (i) a first porous filter element; and (ii) a second porous filter element in contact with the first porous filter element, the second porous filter element arranged downstream of the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one hydrophobic interaction (HIC) layer; binding the nucleic

acid to the at least one AEX layer; eluting the nucleic acid from the at least one AEX layer in high salt; binding the nucleic acid to the at least one HIC layer; and eluting the nucleic acid in low salt buffer.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0006] Figures 1A-1K are drawings showing an aspect of a chromatography device according to an aspect of the invention, having a planar filter configuration. Figures 1A and 1B show, respectively, top and bottom perspective views of an assembled chromatography device having a housing and an annular outlet section engaged with the housing; Figure 1C shows a cross-sectional view of the assembled device shown in Figure 1A, also showing an annular inlet plate and a filter; Figure 1D shows an exploded view of the device, Figures 1F, 1G, and 1H show, respectively, top perspective, bottom perspective, and partial cut-away views of the annular inlet plate; Figures 1I, 1J, and 1K show, respectively, top perspective, bottom perspective, and partial cut-away views of the annular outlet section shown in Figure 1B.

[0007] Figures 2A-2I are drawings of a chromatography device according to another aspect of the invention, having cylindrical hollow filter configuration. Figure 2A shows a top perspective view an assembled chromatography device; Figures 2B and 2C show different cross-sectional views of the device shown in Figure 2A; Figure 2D shows front and rear exploded perspective views of the device; Figures 2E and 2F show, respectively, bottom perspective and cross-sectional views of the inlet section of the device, Figures 2G and 2H show, respectively, side and cross-sectional views of the outlet section of the device; and Figure 2I is a drawing showing a top partial cut-away view of the assembled device.

[0008] Figure 3 is a drawing showing a perspective view of a chromatography device according to another aspect of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0009] In accordance with an aspect of the invention, a chromatography device is provided comprising (a) a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet; (b) a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising (i) first porous filter element; and (ii) a

second porous filter element in contact with the first porous filter element, the second porous filter element arranged downstream of the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one hydrophobic interaction (HIC) layer.

[0010] In one aspect of the chromatography device, the AEX layer(s) and the HIC layer(s) are planar membranes.

[0011] In another aspect of the chromatography device, the AEX layer(s) and the HIC layer(s) are arranged to form a hollow cylindrical filter, e.g., to allow radial flow, wherein the AEX layer surrounds the HIC layer.

[0012] Typical aspects of the chromatography device include at least two AEX layers and at least two HIC layers.

[0013] In another aspect, a method for purifying nucleic acid is provided, the method comprising (a) passing a fluid comprising nucleic acid through a chromatography device comprising a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet; a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising first porous filter element; and a second porous filter element in contact with the first porous filter element, the second porous filter element arranged downstream of the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one hydrophobic interaction (HIC) layer; (b) binding the nucleic acid to the at least one AEX layer; (c) eluting the nucleic acid from the at least one AEX layer in high salt; (d) binding the nucleic acid to the at least one HIC layer; and, (e) eluting the nucleic acid from the at least one HIC layer in low salt buffer.

[0014] Advantageously, a single chromatography device can be used, wherein the method for purification is less complex than conventional methods, while reducing buffer consumption. For example, two purification actions are performed in one operation with a high flow rate (10 membrane volumes/min). Lentivirus and AAV can be eluted with low conductivity buffer from hydrophobic interaction media which aids in their stability

[0015] Each of the components of the invention will now be described in more detail below, wherein like components have like reference numbers.

[0016] Figures 1A-1K are drawings showing an aspect of a chromatography device according to an aspect of the invention, having a planar filter configuration. Figures 1A and 1B show, respectively, top and bottom perspective views of an assembled device having a housing and an annular outlet section engaged with the housing; Figure 1C shows a cross-sectional view of the assembled device shown in Figure 1A, also showing an annular inlet plate and a filter; Figure 1D shows an exploded view of the device, Figures 1F, 1G, and 1H show, respectively, top perspective, bottom perspective, and partial cut-away views of the annular inlet plate; Figures 1I, 1J, and 1K show, respectively, top perspective, bottom perspective, and partial cut-away views of the annular outlet section shown in Figure 1B.

[0017] Figures 1A-1K show an aspect of a chromatography device 100 comprising a filter housing 10 having an annular inner diameter D bounded by a threaded inner side wall 10' (for threadable engagement with a threaded outer wall 12' of an annular outlet section 12), the side wall also including an inwardly facing section 1025 having a lesser inner diameter d1 near the bottom surface 1004, the bottom surface 1004 including an annular projecting ring 1015 as well as an annular shoulder 1020 surrounding a base of an aperture 1010 (for receiving a projection of the annular inlet plate); an annular inlet plate 11 having a projection 1101 including an inlet 11A with optional inner threads 1102, an upper surface 1103, and a lower surface 1104 including an annular projecting ring 1115 providing an inlet chamber 11B (shown with a generally concave configuration, wider toward the filter); and an annular outlet section 12A having an annular outer diameter D2 bounded by a threaded outer wall 12', the outlet section including an inner surface 1204 including a groove 75A including a resilient element 71 such as an o-ring and an inner groove 75B, including an annular projecting ring 1215 providing an outlet chamber 12B (shown with a generally concave configuration, wider toward the filter), and an outlet 12A with inner threads 1202, the device defining a fluid flow path 13 between the inlet and the outlet with a porous filter 150 (shown having a planar configuration) arranged in the filter housing across the fluid flow path, the filter comprising a first porous filter element 151 (that may have at least two layers) and a second porous filter element 152 (that may have at least two layers) in contact with the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one

hydrophobic interaction (HIC) layer, the first porous filter element 151 being arranged upstream of the second porous filter element 152. Preferably, an optional support element 160 such as a mesh or screen is arranged downstream of the porous filter element, e.g., support element 160 contacting the downstream surface of second porous filter element 152. Alternatively, or additionally, a support element, or another support element can be arranged upstream of the porous filter element, e.g., support element contacting the upstream surface of first porous filter element 151.

[0018] While this illustrated aspect shows a threaded inlet and outlet (e.g., for connection with conduits having threaded ends or fittings), aspects of the invention are not so limited, and other connections (e.g., triclamp/sanitary flange connections) are within the skill of the art (*see also*, Figures 2A-2H and 3, showing inlets and outlets each having a connection face with an annular groove for receiving a resilient member such as an o-ring).

[0019] Figures 2A-2I are drawings showing another aspect of a chromatography device 200 (Figure 2A showing a perspective view, and Figures 2B and 2C showing different cross-sectional views of an assembled device) comprising a filter housing 20 having an inlet section 21 with an inlet 21A, the inlet section having an inlet chamber 21B having an annular inner diameter D3 bounded by a threaded inner side wall 20' (for threadable engagement with a threaded outer wall 22' of outlet section 22), the side wall also including an inwardly facing section 2025 having a lesser inner diameter d3 near the bottom surface 2004; and an outlet section 22 with an outlet 22A, having a lower end 25 with a bottom surface 25' (that will contact the top surface of the inwardly facing section 2025 of the inlet section when the inlet and outlet sections are fully engaged), an annular outer diameter D3 bounded by a threaded outer side wall 22' (for threadable engagement with the threaded inner wall 20' of the inlet section), and an annular inner diameter D4 bounded by an inner side wall 24', providing filter chamber 22B having a top wall 26', and a filter cartridge outlet chamber 28B (above/downstream of the filter chamber 22B) for receiving the outlet 273 for filter cartridge 270. In this illustrated aspect (*see*, Figures 2G and 2H), a no-threaded portion of side wall 22' near the lower end 25 includes a groove 27 for receiving a resilient element such as an o-ring (e.g., for improving a fluid tight seal between the inlet and outlet sections); Figure 2I is a drawing showing a top partial cut-away view of the device

[0020] The device 200 defines a fluid flow path 23 (*see*, Figure 2B) between the inlet and the outlet with a filter cartridge 270 including a hollow cylindrical porous filter 250 (*see*, Figure 2I), the filter cartridge and filter being arranged in the filter housing across the fluid flow path, the hollow cylindrical porous filter comprising a first porous filter element 251 and a second porous filter element 252 in contact with the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one hydrophobic interaction (HIC) layer, the first porous filter element 251 being arranged upstream of and surrounding the second porous filter element 252 (each porous filter having a hollow cylindrical configuration; Figure 2I shows the filter 250 with laid-over pleated filter elements 251 and 252) The illustrated filter cartridge has a closed lower end cap 271 (having a lower surface 271A having grooves 272 (for directing flow outwardly) and an upper surface 271B) and an open upper end cap 272 with a hollow extension cartridge outlet 273 (having an outer wall having at least one groove, shown with two grooves 274A, 274B, for receiving resilient elements such as o-rings), and optional support elements such an inner core 260 having a first end 260A and a second end 260B (the ends having respective open chambers 262A, 262B, with a solid section 263 in between) and a side wall with perforations 264 passing through the side wall into the open chamber near the second end 260B (the illustrated inner core has ribs 266 and grooves 267 in the outer surface of the side wall, shown arranged with a continuous angle from the first end to the second end, which can improve channeling liquid toward the outlet), and an outer mesh or cage 261.

[0021] In some aspects, the first porous filter element 251 is a pleated element, and can be a laid-over pleat element. In some aspects, the second porous filter element 252 is a pleated element, and can be a laid-over pleat element. For example, the first and second porous elements can both be pleated elements (e.g., as shown in Figure 2I). Alternatively, the second porous element can wound around the first porous element.

[0022] Preferably, the device 200 includes vents, wherein inlet section 21 includes threaded vent port 290 and threaded vent valve 291, and outlet section 22 includes threaded vent port 292 and threaded vent valve 293.

[0023] In those aspects wherein the device includes vents, the device should be operated in upward flow with the vent(s) open for efficient air displacement from the device.

Accordingly, the inlet side of the device should be oriented face down such that air can be displaced from the device in order to establish uniform fluid flow through the filter. Failure to follow this procedure can result in increased pressure and decreased chromatographic performance, and so it may be desirable to connect a pressure gauge upstream of the device inlet and monitor the pressure. The vent(s) should be opened when the liquid flow is initially established, and closed when all of the air is displaced by liquid. The inlet side of the device should then be oriented face up and liquid passed through the device.

[0024] Figure 3 is a drawing showing a perspective view of another aspect of a chromatography device 300 according to the invention, comprising a filter housing 320 having an inlet section 321 with an inlet 321A, and outlet section 322 (threadably engageable with the inlet section), with threaded vent valves 391 and 393. With the exception of the lengths of the inlet section 321 and the outlet section 322 (and the corresponding lengths of the internal elements (such as those comprising the filter cartridge, the inlet chamber, the filter chamber and the filter cartridge outlet chamber, etc.)), and the diameter of the connections for the inlet 321A and the outlet 322A (diameters are larger in device 300), the structure and operation of device 300 essentially corresponds to that of device 200.

Anion Exchange Layer

[0025] In general, the anionic exchange (AEX) layer is any semi-permeable membrane comprising a base matrix comprising fixed positively charged (cationic) functional groups and mobile anions (counterions) that enable the selective permeability of negatively charged (anionic) species. The base matrix is any suitable material, preferably a hydrophilic material, such as polyether sulfone (PES), cellulose, a methacrylate, a hydrophilic polymer, or any combination thereof.

[0026] In some aspects, the base matrix in the AEX layer comprises a polyether sulfone matrix that is hydrophilic and may or may not include a crosslinked coating. In a preferred aspect of the invention, the polyether sulfone matrix has a crosslinked coating comprising pendant cationic groups, particularly quaternary ammonium groups.

[0027] The crosslinked coating typically comprises a polyamine, a diallylamine copolymer (e.g., a diallylamine copolymer with epoxy groups), and/or an acrylic copolymer, in which one or more of these components includes pendant cationic groups attached directly

or indirectly to the backbone of the polymer. If necessary, the diallylamine copolymer can be reacted with epichlorohydrin to provide epoxy sites. The polyamine can be, e.g., diethylenetriamine, triethylenetetramine, tertaethylenepentamine, pentaethylenehexamine, or polyethylenimine.

[0028] For example, a crosslinked coating can comprise the reaction product of a polyamine (e.g., polyethylenimine) with pendant cationic groups (e.g., quaternary ammonium groups) and optionally a polyalkyleneglycol polyglycidylether. In another example, a crosslinked coating is prepared by crosslinking a composition comprising diallylamine, a diallyldialkylammonium halide, an acrylic monomer having a cationic group (e.g., a quaternary ammonium group), and a crosslinking agent. In another example, a crosslinked coating is prepared by crosslinking a composition comprising a diallylamine copolymer having epoxy groups and pendant cationic groups, a polyethylenimine, and an amine reactive compound having a cationic group (e.g., glycidyl trimethylammonium chloride). In another example, the crosslinked coating comprising a diallylamine copolymer, an acrylic monomer having a quaternary ammonium group, and a crosslinking agent (e.g., an N-(alkoxymethyl)acrylamide). In another example, the crosslinked coating comprises a diallylamine copolymer, an acrylic monomer having a quaternary ammonium group (e.g., an acryloylaminoalkyl trialkylammonium halide or acryloyloxyalkyl trialkylammonium halide), and a crosslinking agent. In another example, the crosslinked coating has pendant cationic groups and comprises an acrylic copolymer comprising a polymerized monomer selected from the group consisting of glycidylalkylacrylate, methacryloyloxyalkyl trialkylammonium halide, and methacryloylaminoalkyl trialkylammonium halide. In another example, the crosslinked coating has pendant cationic groups and comprises an acrylic copolymer linked to pentaethylenehexamine.

[0029] The coating can be crosslinked using any suitable method and/or crosslinking agent. The crosslinking agent is a polyfunctional agent with functional groups that are reactive with a group (e.g., an amino) in the backbone of one more of the polymers in the coating, such as urea, halo, epoxy, isocyanato, carboxyl, or acid chloride. A preferred crosslinking agent is a polyglycidyl compound. An example of a suitable polyglycidyl compound is a polyalkyleneglycol polyglycidylether. Examples of a crosslinking agent include ethyleneglycol diglycidyl ether, 1,4-butanediol diglycidyl ether, N-(isobutoxymethyl)acrylamide, N-(isobutoxymethyl)methacrylamide, epichlorohydrin,

epibromohydrin, and a combination thereof. The crosslinking can take place in a solvent comprising water, at least one organic solvent, or a mixture thereof. If necessary, a catalyst can be used, such as a strong base (e.g., sodium hydroxide, potassium hydroxide).

[0030] In a preferred aspect, the coating solution comprises a copolymer of a diallylamine, diallyldialkylammonium halide, an acrylic monomer having a cationic group (e.g., quaternary ammonium group), and a crosslinking agent.

[0031] The coating composition can be prepared, e.g., by dissolving the monomers (e.g., polyamine) in a suitable solvent (e.g., water, alcohol, such as methanol, ethanol, and propanol, and combinations thereof). The solvent can be present in an amount of from about 40% to about 99%, and preferably in an amount of from about 90% to about 99% by weight of the coating composition. The monomers can be present in an amount of from about 1% to about 5%, and preferably in an amount of from about 1% to about 2.5% by weight of the coating composition. Once the coating solution is applied to the base matrix using any suitable technique (e.g., dipping, spraying, etc.), the solution can be cured to form the crosslinked coating on the base matrix, such that an AEX layer is formed.

[0032] A PES base matrix comprising a crosslinked coating is further described in, for example, U.S. Patent 6,780,327.

[0033] In some aspects, the base matrix in the AEX layer comprises cellulose. Suitable cellulosic polymers include cellulose, regenerated cellulose, cellulose ester (e.g., cellulose acetate, cellulose propionate, cellulose butyrate, cellulose acetate butyrate), cellulose nitrate (also known as nitrocellulose), methylcellulose, ethylcellulose, and combinations thereof (e.g., a mixture of cellulose acetate and cellulose nitrate).

[0034] In some aspects, the base matrix in the AEX layer comprises a methacrylate polymer. Examples of a methacrylate polymer include polymethyl methacrylate and methyl methacrylate copolymer.

[0035] In some aspects, the base matrix in the AEX layer comprises a hydrophilic polymer. Examples of suitable hydrophilic polymers include a polyamide, a polyimide, a polyalkylene oxide, hydrophilic polyurethane, polyvinylpyrrolidone, polyacrylamide, polyacrylic acid, and polyvinyl alcohol. Examples of a polyamide include a nylon, such as

nylon-4,6, nylon-6, nylon-6,6, nylon-11, and/or nylon-12. Examples of a polyimide include an aliphatic polyimide, an aromatic polyimide, or a mixture thereof, such as polyimide derived from poly(amic acid) salt precursor. Examples of a polyalkylene oxide include C₁-C₁₀ polyalkylene oxide, such as polymethylene oxide, polyethylene oxide, polypropylene oxide, and polybutylene oxide. Alternatively, the hydrophilic polymer can be a hydrophobic polymer modified to have hydrophilic functional groups (e.g., hydroxy, amino, aldehyde, carboxy, thio, phosphato, etc.). Such hydrophobic polymers include polyolefins (polyethylene, polypropylene), polystyrene, polycarbonates, fluoropolymers (e.g., polyvinylidene fluoride, polytetrafluoroethylene), and polyether ether ketone (PEEK).

[0036] The pendant cationic functional groups are fixed (e.g., covalently bound) to the base matrix (or a crosslinked coating thereon) and comprise any suitable cationic group, such as ammonium, sulfonium, phosphonium, or a combination thereof. In some embodiments, the cationic functional groups are quaternary ammonium groups, in which the nitrogen is bound to the matrix and three other moieties. In general, the quaternary ammonium group has the structure -NR_3^+ , in which each instance of R is the same or different and is hydrogen, C₁₋₁₀ alkyl, C₃₋₈ cycloalkyl, or aryl. Preferably, the quaternary ammonium group is a trialkylammonium.

[0037] The counteranion of the cationic functional group is not limited, as the counteranion is mobile and does not affect the permeability of the sample to be processed. For example, the counteranion can be hydride, halide (e.g., F⁻, Cl⁻, Br⁻, or I⁻), azide, carbonate, hydrogen carbonate, chlorate, hypochlorite, perchlorate, hydroxide, cyanide, phosphate, sulfate, sulfite, sulfonate, thiosulfate, acetate, formate, oxalate, or a combination thereof. In a preferred aspect, the counteranion is a halide.

[0038] In some embodiments, the fixed cationic functional group can comprise primary amino groups, secondary amino groups, or a combination thereof, which can form a cationic group upon use of the chromatography device. These groups can be the only pendant functional groups or in addition to pendant cationic functional groups.

[0039] The pendant cationic functional group are directly or indirectly bound to the base matrix. Directly bound means a bond exists between the base matrix (or a crosslinked coating thereon) and the pendant cationic functional group. Indirectly bound means a spacer is present between the base matrix (or a crosslinked coating thereon) and the pendant cationic

functional group. The spacer can be of any suitable length or structure (e.g., linear or branched) and typically will include one or more alkylene ($-\text{CH}_2-$) groups and optionally one or more functional groups selected from hydroxy, alkoxy, amino, alkylamino, amido, ester, urea, urethane, and a combination thereof. The spacer group can be of any suitable length. For example, the spacer group can be a group having from 1 to 10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) atoms (e.g., carbon atoms). In an aspect, the spacer group can be from 2 to about 6 (i.e., 1, 2, 3, 4, 5, or 6) atoms long, and preferably 3 carbon atoms long.

[0040] In some aspects, the base matrix (or a crosslinked coating thereon) is polymerized in which at least one of the monomers includes the cationic functional group, such that, when formed, the base matrix already includes the cationic functional group. In other aspects, the base matrix (or a crosslinked coating thereon) is modified after its initial synthesis to add one or more cationic functional groups that are either directly or indirectly bonded, as described herein. In an example, the base matrix comprises pendant amino groups that are quaternized to form pendant ammonium groups.

[0041] The AEX layer can have any suitable pore structure, e.g., a pore size (for example, as evidenced by bubble point, or by KL as described in, for example, U.S. Patent 4,340,479, or evidenced by capillary condensation flow porometry), a mean flow pore (MFP) size (e.g., when characterized using a porometer, for example, a Porvair Porometer (Porvair plc, Norfolk, UK), or a porometer available under the trademark POROLUX (Porometer.com; Belgium)), a pore rating, a pore diameter (e.g., when characterized using the modified OSU F2 test as described in, for example, U.S. Patent 4,925,572), or removal rating media. The pore structure used depends on the size of the particles to be utilized, the composition of the fluid to be treated, and the desired effluent level of the treated fluid.

[0042] For example, the AEX layer can have, for example, an average pore diameter of from about 0.01 μm to about 10 μm . For example, the average pore size is 0.01 μm or more (e.g., 0.01 μm or more, 0.02 μm or more, 0.05 μm or more, 0.1 μm or more, 0.2 μm or more, 0.3 μm or more, 0.5 μm or more, 0.8 μm or more, 1 μm or more, 2 μm or more, or 5 μm or more). Alternatively or in combination, the average pore size is 10 μm or less (e.g., 8 μm or less, 5 μm or less, 2 μm or less, 1 μm or less, 0.8 μm or less, 0.5 μm or less, 0.3 μm or less, 0.2 μm or less, 0.1 μm or less, 0.08 μm or less, 0.05 μm or less, or 0.02 μm or less). Any two of the foregoing endpoints can be used to define a close-ended range, or a single endpoint can

be used to define an open-ended range. In some aspects, the average pore sizes is from about 0.1 μm to about 5 μm , about 0.1 μm to about 2 μm , 0.2 μm to about 5 μm , 0.2 μm to about 1 μm , about 0.1 μm to about 0.2 μm , about 0.5 μm to about 2 μm , about 0.05 μm to about 0.3 μm , 0.1 μm , 2 μm , or 0.45 μm .

[0043] The AEX layer can have any suitable thickness, such as about 50 μm to about 300 μm . For example, the average thickness can be 50 μm or more (e.g., 60 μm or more, 70 μm or more, 80 μm or more, 100 μm or more, 120 μm or more, 140 μm or more, 160 μm or more, 180 μm or more, 200 μm or more, 220 μm or more, 240 μm or more, 260 μm or more, or 280 μm or more). Alternatively or in combination, the average thickness is 300 μm or less (e.g., 280 μm or less, 260 μm or less, 240 μm or less, 220 μm or less, 200 μm or less, 180 μm or less, 160 μm or less, 140 μm or less, 120 μm or less, 100 μm or less, 80 μm or less, 70 μm or less, or 60 μm or less). Any two of the foregoing endpoints can be used to define a close-ended range, or a single endpoint can be used to define an open-ended range. In some aspects, the average pore size is from about 60 μm to about 200 μm , about 80 μm to about 170 μm , 90 μm to about 160 μm , 100 μm to about 200 μm , or about 100 μm to about 200 μm .

Hydrophobic Interaction Layer

[0044] In general, the hydrophobic interaction (HIC) layer is any semi-permeable membrane comprising a base matrix comprising fixed alkyl- and/or phenyl-containing groups. The base matrix is any suitable material, such as polyether sulfone (PES), cellulose, a methacrylate, a hydrophilic polymer, or any combination thereof.

[0045] In some aspects, the base matrix of the HIC layer comprises a polyether sulfone matrix that may or may not include a crosslinked coating. The crosslinked coating can be the same as described with respect to the AEX layer, but in which one or more monomers includes an alkyl- and/or phenyl-containing group or the crosslinked coating is later modified to include alkyl- and/or phenyl-containing groups.

[0046] In some aspects, the base matrix of the HIC layer comprises cellulose. Suitable cellulosic polymers include cellulose, regenerated cellulose, cellulose ester (e.g., cellulose acetate, cellulose propionate, cellulose butyrate, cellulose acetate butyrate), hydrolyzed cellulose ester, cellulose nitrate (also known as nitrocellulose), methylcellulose,

ethylcellulose, and combinations thereof (e.g., a mixture of cellulose acetate and cellulose nitrate).

[0047] In a preferred aspect of the invention, the HIC layer comprises hydrolyzed cellulose acetate comprising fixed alkyl- and/or phenyl-containing groups, preferably phenyl groups. In some embodiments of this aspect, the cellulose acetate has been reinforced with, for example, fiber comprising cellulose (e.g., regenerated cellulose).

[0048] In an example, the base matrix comprises cellulose ester (e.g., cellulose acetate) that is contacted with a solution that swells the cellulose ester matrix and/or hydrolyzes the ester groups to hydroxyl groups to form a cellulose hydrate (hydrolyzed cellulose acetate) matrix. The solution comprises water, one or more organic solvents, such as an alcohol (e.g., ethanol), or a combination thereof. To simultaneously swell and hydrolyze the cellulose ester, the solution requires water and optionally an organic solvent. Otherwise, the swelling and hydrolysis can be performed stepwise. The solution can further comprise one or more additives, such as an acid (e.g., acetic acid), a salt of an acid (e.g., a Group I or II salt of chloride, sulfate, nitrate, phosphate, acetate, formate, etc., such as sodium chloride, sodium sulfate, sodium acetate, etc.), and a combination thereof. If desired, the cellulose hydrate matrix can be crosslinked with one or more crosslinking agents, solvents, and/or catalysts, as described herein.

[0049] In some aspects, the base matrix of the HIC layer comprises a methacrylate polymer. Examples of a methacrylate polymer include polymethyl methacrylate and methyl methacrylate copolymer.

[0050] In some aspects, the base matrix of the HIC layer comprises a hydrophilic polymer. Examples of suitable hydrophilic polymers include a polyamide, a polyimide, a polyalkylene oxide, hydrophilic polyurethane, polyvinylpyrrolidone, polyacrylamide, polyacrylic acid, and polyvinyl alcohol. Examples of a polyamide include a nylon, such as nylon-4,6, nylon-6, nylon-6,6, nylon-11, and/or nylon-12. Examples of a polyimide include an aliphatic polyimide, an aromatic polyimide, or a mixture thereof, such as polyimide derived from poly(amic acid) salt precursor. Examples of a polyalkylene oxide include C₁-C₁₀ polyalkylene oxide, such as polymethylene oxide, polyethylene oxide, polypropylene oxide, and polybutylene oxide.

[0051] The pendant alkyl- and/or phenyl-containing groups are fixed (e.g., covalently bound) to the base matrix (or a crosslinked coating thereon).

[0052] In general, the alkyl portion of the alkyl-containing group is a C₁₋₁₀ alkyl (e.g., C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, or C₁₀ alkyl) that is linear or branched. Examples of the alkyl group include methyl, ethyl, n-propyl, n-butyl, s-butyl, i-butyl, t-butyl, n-pentyl, i-pentyl, neo-pentyl, t-pentyl, hexyl, heptyl, octyl, nonyl, and decyl. The alkyl-containing group can be unsubstituted or substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6) substituents, such as C₃₋₈ cycloalkyl (C₃, C₄, C₅, C₆, C₇, or C₈ cycloalkyl), halo (e.g., F, Br, Cl, and/or I), halo-C_{1-C₁₀} alkyl, nitro, C₂₋₆ alkenyl, aryl (e.g., phenyl, naphthyl), and a combination thereof. The substituents can be the same or different, but preferably they are the same. In some embodiments, the alkyl-containing group is n-butyl, hexyl, or octyl.

[0053] In general, the phenyl portion of the phenyl-containing group is unsubstituted or substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6) substituents, such as C₁₋₁₀ alkyl (e.g., C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, or C₁₀ alkyl), C₃₋₈ cycloalkyl (C₃, C₄, C₅, C₆, C₇, or C₈ cycloalkyl), halo (e.g., F, Br, Cl, and/or I), nitro, C₂₋₆ alkenyl, aryl (e.g., phenyl, naphthyl), and a combination thereof. The substituents can be the same or different, but preferably they are the same. When the phenyl is substituted with a substituent, the aromatic ring hydrogen is replaced with the substituent and this can take place at any of the available hydrogens, e.g., 2-, 3-, 4-, 5-, and/or 6-position wherein the 1-position is the point of attachment of the phenyl group to the base matrix, either directly or indirectly. In some embodiments, the phenyl-containing group is phenyl.

[0054] In any of the aspects above, whenever a range of the number of atoms in a structure is indicated (e.g., a C₁₋₁₀, C₁₋₈, C₁₋₆, C₁₋₄, etc.), it is specifically contemplated that any sub-range or individual number of carbon atoms falling within the indicated range also can be used. Thus, for instance, the recitation of a range of 1-8 carbon atoms (e.g., C_{1-C₈}), 1-6 carbon atoms (e.g., C_{1-C₆}), 1-4 carbon atoms (e.g., C_{1-C₄}), 1-3 carbon atoms (e.g., C_{1-C₃}), or 2-8 carbon atoms (e.g., C_{2-C₈}) as used with respect to any chemical group (e.g., alkyl, cycloalkyl, etc.) referenced herein encompasses and specifically describes 1, 2, 3, 4, 5, 6, 7, and/or 8 carbon atoms, as appropriate, as well as any sub-range thereof (e.g., 1-2 carbon atoms, 1-3 carbon atoms, 1-4 carbon atoms, 1-5 carbon atoms, 1-6 carbon atoms, 1-7 carbon atoms, 1-8 carbon atoms, 2-3 carbon atoms, 2-4 carbon atoms, 2-5 carbon atoms, 2-6 carbon

atoms, 2-7 carbon atoms, 2-8 carbon atoms, 3-4 carbon atoms, 3-5 carbon atoms, 3-6 carbon atoms, 3-7 carbon atoms, 3-8 carbon atoms, 4-5 carbon atoms, 4-6 carbon atoms, 4-7 carbon atoms, 4-8 carbon atoms, etc., as appropriate).

[0055] The pendant alkyl- and/or phenyl-containing groups are directly or indirectly bound to the base matrix (or a crosslinked coating thereon). Directly bound means a bond exists between the base matrix (or a crosslinked coating thereon) and the pendant alkyl- and/or phenyl-containing group. Indirectly bound means a spacer is present between the base matrix (or a crosslinked coating thereon) and the pendant alkyl- and/or phenyl-containing group. The spacer can be of any suitable length or structure (e.g., linear or branched) and typically will include one or more alkylene ($-\text{CH}_2-$) groups and optionally one or more functional groups, such as hydroxy, halo, alkoxy, amino, alkylamino, amido, ester, urea, urethane, and a combination thereof. The spacer group can be of any suitable length. For example, the spacer group can be a group having from 1 to 10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) atoms (e.g., carbon atoms). In an aspect, the spacer group can be from 2 to about 6 (i.e., 1, 2, 3, 4, 5, or 6) atoms long, and preferably 3 carbon atoms long.

[0056] In some aspects, the base matrix (or a crosslinked coating thereon) is polymerized in which at least one of the monomers includes the alkyl- and/or phenyl-containing group, such that, when formed, the base matrix (or a crosslinked coating thereon) already includes the alkyl- and/or phenyl-containing group. In other aspects, the base matrix (or a crosslinked coating thereon) is modified after its initial synthesis to add one or more alkyl- and/or phenyl-containing groups that are either directly or indirectly bonded, as described herein. In an example, the base matrix comprises one or more functional groups, such as hydroxyl, epoxide, and/or aldehyde groups, that can be modified to add the alkyl- and/or phenyl-containing group using, for example, alkyl glycidyl ether, alkylamine (e.g., butylamine, hexylamine, octylamine), phenyl glycidyl ether, or aniline.

[0057] The HIC layer can have any suitable pore structure, e.g., a pore size (for example, as evidenced by bubble point, or by KL as described in, for example, U.S. Patent 4,340,479, or evidenced by capillary condensation flow porometry), a mean flow pore (MFP) size (e.g., when characterized using a porometer, for example, a Porvair Porometer (Porvair plc, Norfolk, UK), or a porometer available under the trademark POROLUX (Porometer.com; Belgium)), a pore rating, a pore diameter (e.g., when characterized using the modified OSU

F2 test as described in, for example, U.S. Patent 4,925,572), or removal rating media. The pore structure used depends on the size of the particles to be utilized, the composition of the fluid to be treated, and the desired effluent level of the treated fluid.

[0058] For example, the HIC layer can have for example, an average pore diameter of from about 0.1 μm to about 20 μm . For example, the average pore size is 0.1 μm or more (e.g., 0.2 μm or more, 0.3 μm or more, 0.5 μm or more, 0.8 μm or more, 1 μm or more, 2 μm or more, 3 μm or more, 5 μm or more, 8 μm or more, 10 μm or more, 12 μm or more, 15 μm or more, or 18 μm or more). Alternatively or in combination, the average pore size is 20 μm or less (e.g., 18 μm or less, 15 μm or less, 12 μm or less, 10 μm or less, 8 μm or less, 5 μm or less, 3 μm or less, 2 μm or less, 1 μm or less, 0.8 μm or less, 0.5 μm or less, 0.3 μm or less, or 0.2 μm or less). Any two of the foregoing endpoints can be used to define a close-ended range, or a single endpoint can be used to define an open-ended range. In some aspects, the average pore size is from about 0.1 μm to about 15 μm , about 0.1 μm to about 10 μm , 0.2 μm to about 5 μm , 0.2 μm to about 1 μm , about 1 μm to about 5 μm , about 2 μm to about 4 μm , 3 μm , or 0.45 μm .

[0059] The HIC layer can have any suitable thickness, such as about 50 μm to about 400 μm . For example, the average thickness can be 50 μm or more (e.g., 60 μm or more, 70 μm or more, 80 μm or more, 100 μm or more, 120 μm or more, 140 μm or more, 160 μm or more, 180 μm or more, 200 μm or more, 220 μm or more, 240 μm or more, 260 μm or more, 280 μm or more, 300 μm or more, 320 μm or more, 340 μm or more, 360 μm or more, or 380 μm or more). Alternatively or in combination, the average thickness is 400 μm or less (e.g., 380 μm or less, 360 μm or less, 340 μm or less, 320 μm or less, 300 μm or less, 280 μm or less, 260 μm or less, 240 μm or less, 220 μm or less, 200 μm or less, 180 μm or less, 160 μm or less, 140 μm or less, 120 μm or less, 100 μm or less, 80 μm or less, 70 μm or less, or 60 μm or less). Any two of the foregoing endpoints can be used to define a close-ended range, or a single endpoint can be used to define an open-ended range. In some aspects, the average pore size is from about 60 μm to about 300 μm , about 80 μm to about 270 μm , 90 μm to about 260 μm , 100 μm to about 250 μm , or about 250 μm .

Both AEX and HIC Layers

[0060] The AEX and HIC membranes can be provided by any suitable method, such as purchased commercially or synthesized using methods known in the art. For example,

commercial AEX membranes include the MUSTANG™ Q series (Pall, Port Washington NY) and SARTOBIND Q™ or SARTOBIND STIC™ (Sartorius AG, Göttingen, Germany). Commercial HIC membranes include SARTOBIND™ Phenyl (Sartorius AG, Göttingen, Germany).

[0061] The hydrophilicity and hydrophobicity of the polymers forming the base matrix or the layers can be measured, if necessary, by any suitable method. For example, a drop of water can be placed on a film or pressed pellet of the polymer, and the contact angle (i.e., the angle of the liquid meeting the solid surface) of the side view of the water droplet can be measured using, for example, a contact angle goniometer. The sessile drop measurement is the most common method of measuring the contact angle. In general, a contact angle less than 90° is considered a hydrophilic material, whereas a contact angle greater than 90° is considered a hydrophobic material.

[0062] Alternatively, the layers can have any desired critical wetting surface tension (CWST, as defined in, for example, U.S. Patent 4,925,572). The porous membrane can have any desired critical wetting surface tension (CWST, as defined in, for example, U.S. Patent 4,925,572). The CWST can be selected as is known in the art, e.g., as additionally disclosed in, for example, U.S. Patents 5,152,905, 5,443,743, 5,472,621, and 6,074,869.

The Chromatography Device

[0063] In a preferred aspect, the chromatography device 100, 200 comprises a porous filter 150 (planar configuration), 250 (radial configuration) comprising an upstream first porous filter membrane 151, 251 comprising at least one AEX layer comprising a polyether sulfone matrix with a crosslinked coating comprising pendant quaternary ammonium groups, and a downstream second porous filter membrane 152, 252 comprising at least one HIC layer comprising crosslinked cellulose hydrate comprising pendant phenyl groups.

[0064] Chromatography devices according to aspects of the invention can have any number of AEX layers and any number of HIC layers. Typically, the devices have at least two AEX layers and two HIC layers; in some aspects, having, for example, 3, 4, 5, 6, 7, 8, 9, or more, AEX layers and 3, 4, 5, 6, 7, 8, 9, or more, HIC layers. Chromatography devices according to aspects of the invention can have different numbers of AEX layers compared to HIC layers, e.g., a greater number of AEX layers than HIC layers, or vice versa.

[0065] The chromatography device can include additional elements, layers, or components, that can have different structures and/or functions, e.g., at least one of any one or more of the following: prefiltration, support, drainage, spacing and cushioning. Illustratively, the filter can also include at least one additional element such as a mesh and/or a screen. In a preferred aspect, the device includes a downstream support element 160, 260.

[0066] In accordance with aspects of the invention, the filter and filter elements can have a variety of configurations, including planar (e.g., as shown in Figure 1), pleated, and hollow cylindrical (e.g., as shown in Figures 2A, 2B, and 2I).

[0067] The chromatography device can be sterilizable. Any filter housing of suitable shape and providing at least one inlet and at least one outlet may be employed. The filter housing can be fabricated from any suitable rigid impervious material, including any impervious thermoplastic material, which is compatible with the fluid being processed. In a preferred aspect, the housing is fabricated from a polymer, such as an acrylic, polypropylene, polystyrene, or a polycarbonated resin.

Method of Purification

[0068] In an aspect, a method of purification comprises (a) passing a fluid comprising nucleic acid through a chromatography device comprising a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet; a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising a first porous filter element; and a second porous filter element in contact with the first porous filter element, wherein the first porous filter element comprises at least one anionic exchange (AEX) layer, and the second porous filter element comprises at least one hydrophobic interaction (HIC) layer; (b) binding the nucleic acid to the AEX layer; (c) eluting the nucleic acid from the AEX layer in high salt; (d) binding the nucleic acid to the HIC layer; and, (e) eluting the nucleic acid from the HIC layer in low salt buffer.

[0069] For example, the nucleic acid is captured on the AEX layer under binding conditions (pH above isoelectric point and conductivity below that required for binding). The membrane is washed with binding buffer and eluted from the AEX layer with salt solution (such Sodium Citrate or Ammonium Sulfate) and bound onto the HIC layer. The HIC

membrane is washed with salt buffer and eluted with AEX binding buffer (HIC elution buffer).

[0070] For purification of enveloped viruses such as lentivirus, the biomolecule of interest is dissolved in buffer having a pH higher than the isoelectric point (thus negatively charged) and conductivity below what is needed for elution from AEX layer, and passed thorough the chromatography device at 10 membrane volumes/min flow rate, such that the biomolecule binds to the AEX layer. A wash with binding buffer is executed and the biomolecule is eluted from the AEX layer using a buffer of high enough conductivity to elute the biomolecule (causing desorption by equilibrating - elution buffer) and to bind the biomolecule to the HIC layer. The HIC layer bound with the biomolecule is washed with elution buffer and eluted with a buffer having a pH higher than the isoelectric point of the biomolecule and low conductivity.

[0071] Alternative buffers such as, for example, MES, HEPES, MOPS, TAPSO, BES, PIPES, ACES, can be used. Sodium Chloride, Magnesium Chloride at concentration of 1.5 M minimum can be used as alternative to Sodium Citrate or Ammonium Sulfate.

[0072] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0073] This example demonstrates lentivirus purification using a chromatography device according to an aspect of the invention.

[0074] A chromatography device is provided as generally shown in Figure 1C. The chromatography device includes 4 layers of AEX membrane and 4 layers of HIC membrane, minimum 0.1 cm in diameter, fitted into a filter holder with AEX membrane on the inlet (upstream) side and the HIC membrane on the outlet (downstream) side, and is washed with 10 mL of 1 M NaOH solution then equilibrated with 25 mL of 25 mM Tris pH 8, 100 mM NaCl (prepared from 25 mL of 1 M Tris and 25 mL of 5 M NaCl added to 950 mL of DI water).

[0075] 10 mL of clarified lentivirus harvest from HEK293 cells containing approximately 1.6×10^6 vectors/mL with conductivity of about 15 mS/cm is flown through chromatography

device at anywhere from 1 to 10 membrane volumes/min flow rate. The membranes are washed with 4 mL of AEX Equilibration buffer (25mM Tris pH8 100 mM NaC and eluted from AEX media/bound on HIC media with 4 mL of 1.5 M Sodium Citrate pH 8.0 (prepared from adding 441.15 g of Sodium Citrate dihydrate and 25 mL of 1 M Tris pH 8 into in total 1 L of DI water). The device is washed with 4 mL of elution buffer. The HIC membrane is eluted with 4 mL of AEX equilibration buffer (25 mM Tris, pH 8 100 mM NaCl) (a low salt buffer) to contain approximately 8.3×10^5 Vectors/mL free of DNA and host-cell protein (a 15% yield measured by functional titer assay after two cycles of freeze and thaw). The conductivity of eluted lentivirus harvest is about 40 mS/cm.

[0076] The lentivirus is recovered in high purity, free of host cell proteins and DNA, without requiring buffer dilution.

EXAMPLE 2

[0077] This example demonstrates lentivirus purification using a chromatography device according to an aspect of the invention, wherein, in contrast with Example 1, the yield is measured without freeze and thaw.

[0078] The procedure summarized in Example 1 is generally followed, except that the yield is measured following the manufacturer's instructions for using the Gyrolab[®] p24 kit for Lentivirus titer determination (2021; Gyros Protein Technologies).

[0079] The yield (using an ELISA assay measuring free surface p24 protein) is over 30%.

EXAMPLE 3

[0080] This example describes a chromatography device according to another aspect of the invention.

[0081] A chromatography device is provided as generally shown in Figures 2B and 2I. The chromatography device includes 8 layers of AEX pleated membrane and 8 layers of pleated HIC membrane, with the AEX membrane wound around the HIC membrane, fitted into the housing with AEX membrane on the inlet (upstream) side.

[0082] In one aspect, the eluted lentivirus is analyzed to determine to lentivirus recovery (Vectors/mL) by Transduction Units (TU) assay using Fluorescence-Activated Cell Sorting

(FACS) after freeze and thaw free of DNA and host-cell protein, without requiring buffer dilution.

[0083] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0084] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0085] Preferred aspects of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred aspects may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of

the subject matter recited in the claims appended hereto as permitted by applicable law.

Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A chromatography device for purification of enveloped viruses, comprising:
 - (a) a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet;
 - (b) a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising:
 - (i) a first porous filter element; and
 - (ii) a second porous filter element in contact with the first filter element, the second porous filter element arranged downstream of the first porous filter element, wherein the first filter element comprises at least one anionic exchange (AEX) membrane, and the second filter element comprises at least one hydrophobic interaction (HIC) membrane.
2. The chromatography device of claim 1, wherein the enveloped virus is a lentivirus.
3. The chromatography device of claim 1, wherein the AEX membrane and the HIC membrane are planar membranes.
4. The chromatography device of claim 1, wherein the porous filter comprises a hollow cylindrical filter wherein the AEX membrane surrounds the HIC membrane.
5. The chromatography device of any one of claims 1-4, wherein the porous filter includes two or more AEX membranes and two or more HIC membranes.
6. A method for purifying enveloped viruses, the method comprising:
 - (a) passing a fluid comprising enveloped viruses through a chromatography device comprising a filter housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet; a porous filter arranged in the filter housing across the fluid flow path, the porous filter comprising a first porous filter element; and a second porous filter element in contact with the first filter element, the second porous filter element arranged downstream of the first porous filter element, wherein the first filter element comprises at least one anionic exchange (AEX) membrane, and the second filter element comprises at least one hydrophobic interaction (HIC) membrane;

- (b) binding the enveloped viruses to the at least one AEX membrane;
 - (c) eluting the enveloped viruses from the at least one AEX membrane in high salt;
 - (d) binding the enveloped viruses to the at least one HIC membrane; and,
 - (e) eluting the enveloped viruses from the at least one HIC membrane in low salt buffer.
7. The method of claim 6, wherein the enveloped virus is a lentivirus.