FILTER STRUCTURE WITH ENHANCED DIRT HOLDING CAPACITY

FIELD OF THE INVENTION

[001] The present invention relates generally to fluid filtration devices, and more particularly to a fluid filtration device incorporating a composite media filter structure, as well as methods of using and making such devices and structures.

BACKGROUND OF THE INVENTION

[002] Fluid filters designed for removal of unwanted particulates and contaminants are susceptible to clogging due to the fact that as particles and contaminants become trapped in the filter medium, fluid pathways become closed or restricted. The capacity or service life of a filter is related to its ability to flow fluid at a rate above a minimum value, or to maintain a desired flow rate at a pressure below a maximum value.

[003] One approach towards increasing the required dirt-holding capacity or service life of a filter is to protect the final filter with a prefilter having a coarser structure. A prefilter will generally have a particle size removal rating larger than that of the final filter. Because the coarser structure of the prefilter will remove relatively large particles that would otherwise clog the final filter, the service life of the final filter will be increased. In addition, owing to its coarser structure, the prefilter will not clog as readily as the final filter against the same particulate challenge. Therefore, the combined prefilter and final filter allows for a longer filter service life as compared to the final filter by itself.

[004] The addition of a coarser structure prefilter to a filter device does however have drawbacks, such as the cost associated with supporting and housing the additional prefilter material in a device. The addition of a prefilter can increase the total space occupied by the filtration system. This increase in filter volume is considered a drawback since compactness is a desired property of a filtration system.

[005] It is therefore desirable to develop a fluid filtration structure having an increased service life and required dirt-holding capacity, capable of removing relatively large contaminants and particles that would otherwise clog a final filter. It is also desirable to lower the manufacturing cost of a fluid filtration device, as well as reduce the overall size requirements of such a device, by incorporating a fluid filtration structure having these advantageous properties.

SUMMARY OF THE INVENTION

[006] In response to the above needs and problems associated with prefilter and final filter assemblies, the present invention provides fluid filtration structures comprising a composite filter media having a coarse porous interlayer located between at least two fiber containing porous filter layers. In certain embodiments, the at least two porous filter layers are made from electropsun polymeric nanofibers each having a different fiber diameter.

[007] Another embodiment of the invention includes a composite filter media containing one or more polymeric non-woven coarse interlayers located between the at least two polymeric fiber containing filter layers or mats.

[008] Other embodiments of the invention include a composite filter media having (i) a first filter mat comprising first polymeric fibers having a first fiber diameter, (ii) a second filter mat comprising second polymeric fibers having a second fiber diameter, and (iii) a coarse interlayer located between the first and second filter mats, wherein the first fiber diameter is different from the second fiber diameter, and each filter mat has a different pore size rating as determined by the actual pore sizes.

[009] In another embodiment of the invention, the first and second polymeric fibers in the composite filter media are nanofibers.

[010] In another embodiment of the invention, the first and second polymeric nanofibers are electrospun nanofibers.

[011] In certain embodiments, the invention provides a composite filter media containing an interlayer having a coarser pore size when compared to either of the first or second filter layers, wherein the mean pore size of the interlayer is approximately 2 to 100 times larger than the mean pore size of the first or second fiber containing filter layers on either side of the interlayer, such that the resulting composite filter media has an increased dirt holding capacity compared to filter layers that are layered directly over each other without the presence of the coarser interlayer therebetween.

[012] In still other embodiments, the invention provides a fluid filtration device housing a fluid filtration structure comprising a composite media made from one or more polymeric non-woven coarse interlayers located between at least two polymeric fiber containing filter mats.

[013] In another embodiment of the invention, the at least two polymeric fibers in the composite filter media are nanofibers.

[014] In another embodiment of the invention, the nanofibers are electrospun nanofibers.

[015] In still other embodiments, the invention provides methods of using and methods of making a fluid filtration device housing a fluid filtration structure comprising a composite media made from one or more polymeric non-woven coarse interlayers located between at least two polymeric fiber containing filter mats.

[016] Another embodiment of the invention provides methods of using and methods of making the composite media wherein the polymeric fibers are nanofibers.

[017] Another embodiment of the invention provides methods of using and methods of making the composite media wherein the polymeric nanofibers are electrospun nanofibers.

[018] Additional features and advantages of the embodiments of the invention will be set forth in the detailed description, claims, and drawings which follow. The specific embodiments described herein are offered by way of example only and are not meant to be limiting in any way. Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Further aspects and advantages of the present invention will become apparent from the description hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[019] Figs. 1A, 1B, and 1C depict cross-sectional views of composite media structures in accordance with certain embodiments of the invention, and Fig. 1D depicts a cross-sectional view of a prior art multilayer filter media with no interlayers located between each filter layer;

[020] Figs. 2 is a bar graph showing the effect of the filter layer arrangement and the presence or absence of an interlayer on throughput capacity of a filter device in accordance with certain embodiments;

[021] Fig. 3 is a bar graph showing the effect of the filter layer arrangement and the presence or absence of an interlayer on throughput capacity of a filter device in accordance with certain embodiments; and

[022] Fig. 4 is a bar graph of showing the effect of the filter layer arrangement and the presence or absence of an interlayer on throughput capacity of a filter device in accordance with certain embodiments.

[023] Fig. 5 is a bar graph of showing the effect of the filter layer arrangement and the presence of an interlayer on throughput capacity of a filter device in accordance with certain embodiments.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[024] Unless otherwise indicated, all numbers expressing quantities of ingredients, cell culture, treatment conditions, and so forth used in the specification, including claims, are to be understood as being modified in all instances by the term “about.” Accordingly, unless otherwise indicated to the contrary, the numerical parameters are approximations and may vary depending upon the desired properties sought to be obtained by the present invention.

[025] For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities of ingredients, percentages or proportions of materials, reaction conditions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about".

[026] Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[027] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass all subranges subsumed therein.

[028] 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.

[029] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context.

[030] 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.

[031] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only and are not meant to be limiting in any way. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

[032] Before describing the present invention in further detail, a number of terms will be defined. Use of these terms does not limit the scope of the invention but only serve to facilitate the description of the invention. Additional definitions may be set forth throughout the detailed description.

I. DEFINITIONS

[033] The use of the terms "a" and "an" and "the" 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.

[034] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series.

[035] As used herein, the term "nanofibers" refers to fibers having diameters varying from a few nanometers up to 1,000 nanometers.

[036] As used herein, the terms “fluid filtration structure”, “composite media”, “composite medium”, "filter medium" or "filter media" refer to a collection of materials through which a fluid carrying the product of interest as well as particles and contaminants passes, wherein the particles and contaminants are deposited in or on the media.

[037] As used herein, the term “permeability” refers to the rate at which a volume of fluid passes through a filtration medium of a given area at a given pressure drop across the filter.

Common units of permeability are liters per square meter per hour for each psi of pressure drop, abbreviated as LMH/psi.

[038] The term “electrospinning” or “electrospun”, as used herein, refers to an electrostatic spinning process of producing nanofibers from a polymer solution or melt by applying an electric potential to such solution. The electrostatic spinning process for making an electrospun nanofiber mat for a filtration medium, including a suitable apparatus for performing the electrostatic spinning process is disclosed in International Publication Nos. WO 2005/024101, WO 2006/131081, and WO 2008/106903, each fully incorporated herein by reference, and each assigned to Elmarco S.R.O., of Liberec, Czech Republic.

[039] The term “nanofiber mat” as used herein, refers to an assembly of multiple nanofibers, such that the thickness of the mat is typically at least about 10 times greater than the diameter of a single fiber in the mat. The nanofibers can be arranged randomly in the mat, or aligned along one or multiple axes.

[040] The term “biopharmaceutical preparation” or “sample” as used herein, refers to any liquid composition containing a product of interest (*e.g.*, a therapeutic protein or an antibody) and unwanted components, particles, and contaminants or particles, such as protein aggregates (*e.g.*, high molecular weight aggregates of the product of interest).

II. EXEMPLARY FLUID FILTRATION STRUCTURES

[041] Fig. 1A depicts an exemplary embodiment of the fluid filtration structure (20) including a composite media comprising at least one coarse porous nonwoven interlayer (25) located between first (26) and second (28) electropsun polymeric fiber containing filter mats.

[042] Fig. 1B depicts an exemplary embodiment of the fluid filtration structure (30) including a composite media comprising at least one coarse porous nonwoven interlayer (35) located between first (32) and second (36) electropsun polymeric fiber containing filter mats, and additional electropsun polymeric fiber containing mats (34, 38) each having a different pore size rating compared to the first and second fiber containing mats. Mats (36, 38) and (32, 34) are layered respectively over each other to form an asymmetric structure. Creating asymmetry in such mat structures improves the dirt holding capacity of the composite media filters. In effect, the coarser portion of the fluid filtration structure that faces the challenge stream acts as prefilter for the finer portion of the structure that is designed to prevent passage of particles larger than a specified size (the "retentive" layer).

[043] Fig. 1C depicts an exemplary embodiment of the fluid filtration structure (40) including a composite media comprising at least one coarse porous nonwoven interlayers (45) located between first (42) and second (44); third (46) and second (44); and fourth (48) and third (46) electropsun polymeric fiber containing filter mats.

III. EXEMPLARY NANOFIBER POLYMERIC MATERIALS

[044] Certain exemplary embodiments of polymers suitable for use as the electrospun nanofibers of the invention include thermoplastic and thermoset polymers. Nonlimiting examples of suitable polymers include nylon, polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, poly(vinyl chloride), poly(vinyl alcohol), poly(vinyl acetate), poly(vinylidene fluoride), poly(vinyl butylene), copolymers, derivative compounds, blends and combinations thereof. Suitable polyamide condensation polymers, include nylon-6; nylon-4,6; nylon-6,6;nylon 6,6-6,10; copolymers of the same, and other linear generally aliphatic nylon compositions and the like.

[045] The term “nylon” as used herein includes nylon-6, nylon-6,6, nylon 6,6-6,10, and copolymers, derivative compounds, blends and combinations thereof.

IV. EXEMPLARY METHODS OF FORMING POROUS FIBROUS MATS

[046] Certain exemplary embodiments of the fibrous porous mats are made by depositing nanofiber(s) from a nylon solution. Such nanofiber filter mats have a preferred basis weight of between about 0.1 g/m^2 and about 10 g/m^2, as measured on a dry basis, (i.e., after the residual solvent has evaporated or otherwise been removed).

[047] In other exemplary embodiments, the nylon is dissolved in a mixture of solvents including, but not limited to, formic acid, sulfuric acid, acetic acid, 2,2,2-trifluoroethanol, 2,2,2,3,3,3-hexafluoropropanol, and water.

[048] In other exemplary embodiments, the nylon solution is prepared by dissolving dry nylon polymers in one group of solvents (i.e., first preparing a stock solution) then adding other solvents to make the solution ready for electrospinning.

[049] In other exemplary embodiments, the nylon polymers (i.e., starting) are partially hydrolyzed over the course of solution preparation, such that the average molecular weight of the

partially hydrolyzed nylon polymers (i.e., ending) are less than the average molecular weight of the starting nylon polymers.

[050] In certain exemplary embodiments, a single or multilayered porous coarse nonwoven interlayer substrates or supports are arranged on a moving collection belt to collect and combine with the electrospun nanofiber filter layer, forming a composite filtration media structure.

[051] One preferred method for generating porous filter membranes for particulate removal is to place fibers of a controlled size on a porous mat substrate such that the spaces among the fibers constitute the filter pores. The average pore diameter is related to the fiber diameter according to Equation 1 below:

[052] $$d = \frac{d_f\,\varepsilon}{(1-\varepsilon)}$$

[053] Equation 1 (where d is the average pore diameter, d_f is the fiber diameter, and Ɛ is the porosity of the mat)

[054] A composite graded fiber filter structure can be formed by layering filter mats of different controlled fiber sizes on each other. In this way, an essentially asymmetric porous structure can be formed. As provided above, this asymmetric porous structure allows for a higher dirt holding capacity than a symmetric structure.

[055] Fig. 1D depicts a conventional graded fiber filter mat arrangements (50) wherein the fiber containing filter mat layers are typically laid directly against each other. (52, 54, 56, 58)

[056] However, it has been surprisingly found that by separating the fiber filter mat layers with a porous interlayer having a much coarser pore size than any of the fiber filter mat layers, clogging resistance in such fiber filtration structures are substantially improved compared to filtration structures where the fiber filter mat layers are directly in contact with each other (i.e., no porous support interlayer located between each filtration mat layer). (Fig. 1A)

[057] The mechanism that allows for the higher clogging resistance associated with the coarse interlayer separating the individual fiber mat layers is not well understood, although it can be speculated that particles may have an increased tendency to become trapped at the interface between fiber mat layers of different fiber sizes. Regardless of the actual mechanism, the addition of a porous coarse interlayer results in reduced filter clogging and therefore increased service life of the filter.

[058] Other considerations for choosing a multilayered filtration structure include economics and convenience of media and device manufacturing, as well as ease of sterilization and validation. The preferred filter layer configuration for the filtration medium is often selected based on practical considerations.

V. EXEMPLARY INTERLAYER SUBSTRATES FOR COLLECTING THE NANOFIBERS

[059] The fluid filter layers collected or deposited on the coarse interlayer are either a single or multi-layer configuration.

[060] Examples of single or multilayered porous coarse interlayers include, but are not limited to, spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens and combinations thereof.

[061] Exemplary embodiments of the porous coarse interlayer are made from synthetic or natural polymeric materials. Thermoplastics are a useful class of polymers for this use. Thermoplastics include but are not limited to polyolefins such as polyethylenes, including ultrahigh molecular weight polyethylenes, polypropylenes, sheathed polyethylene/polypropylene fibers, PVDF, polysulfone, polyethersulfones, polyarylsulphones, polyphenylsulfones, polyvinyl chlorides, polyesters such as polyethylene terephthalate, polybutylene terephthalate and the like, polyamides, acrylates such as polymethylmethacrylate, styrenic polymers and mixtures of the above. Other synthetic materials include celluloses, epoxies, urethanes and the like.

[062] Suitable porous coarse interlayer substrates include porous non-woven substrates, (i.e., those having pore sizes from about (1) µm to about (100 µm.

[063] The porous coarse interlayer material can be hydrophilic nonwovens or hydrophobic nonwovens and include, but are not limited to, polyolefins, polypropylene, polyvinylidene fluoride, polytetafluoroethylene, polysulfones, polycarbonates, polyesters, polyacrylates, and polymethacrylates.

[064] The porous coarse interlayer has two major opposing surfaces or sides (i.e., opposing first and second sides) associated with a geometric and/or physical structure and/or shape of the interlayer. When the composite media is being used in sample filtration, fluid will flow from one side (surface) through the interlayer substrate and through the opposing other side (surface).

[065] The thickness dimension between the two opposing surfaces of the interlayer is porous. This porous region has a surface area associated with the pores. In order to prevent confusion related to the terms "surface", "surfaces", or "surface area," or similar usages, the

geometric surfaces will be referred to as external or facial surfaces or as sides. The surface area associated with the pores will be referred to as internal or porous surface area.

[066] Porous material of the interlayer comprises pores which are empty space, and the solid matrix or skeleton which makes up the physical embodiment of the interlayer material. For example, in a non-woven interlayer, the randomly oriented fibers make up the matrix and give the interlayer its form.

[067] When the composite media includes a coating or covering on a surface of the composite media means that the internal and external surfaces are coated so as to not completely block the pores, that is, to retain a significant proportion of the porous structure for convective flow. In particular, for the internal surface area, coating or covering means that the porous matrix is coated or covered, leaving a significant proportion of the pores open.

VI. EXEMPLARY TEST METHODS PERFORMED

[068] "Basis weight" is determined by ASTM D-3776, which is incorporated herein by reference and reported in g/m^2.

[069] "Porosity" was calculated by dividing the basis weight of the sample in g/m^2 by the polymer density in g/cm^3, by the sample thickness in micrometers, multiplying by 100, and subtracting the resulting number from 100, i.e., porosity =100 - [basis weight/(density x thickness) x 100].

[070] Fiber diameter was determined as follows: a scanning electron microscope (SEM) image was taken at 60,000 times magnification of each side of a nanofiber mat sample. The diameter of ten (10) clearly distinguishable nanofibers were measured from each SEM image and recorded. Defects were not included (i.e., lumps of nanofibers, polymer drops, intersections of nanofibers). The average fiber diameter of each side of the nanofiber mat sample was calculated. The measured diameters also include a metal coating applied during sample preparations for SEM. It was established that such coating adds approximately 4 to 5 nm to the measured diameter. The diameters reported here have been corrected for this difference by subtracting 5 nm from the measured diameter.

[071] Thickness was determined by ASTM D1777-64, which is incorporated herein by reference, and is reported in micrometers (or microns) and is represented by the symbol "μm".

[072] "Permeability" is the rate at which fluid passes through the composite filter media samples of a given area at a given pressure drop, and measured by passing deionized water

through composite filter media samples having a diameter of 25 (3.5 cm^2 filtration area) mm. The water was forced through the composite filter media samples using hydraulic pressure (water head pressure) or pneumatic pressure (air pressure over water).

[073] The "effective pore size" of an electrospun mat can be measured using conventional membrane techniques such as bubble point, liquid-liquid porometry, and challenge test with particles of certain sizes. It is known that the effective pore size of a fibrous mat generally increases with the fiber diameter and decreases with porosity.

[074] Membrane manufacturers assign nominal pore size ratings to commercial membrane filters, which usually indicate meeting certain retention criteria for particles or microorganisms rather than geometrical size of the actual pores.

[075] The invention will be further clarified by the following examples of exemplary embodiments which are intended to be exemplary of the invention.

[076] Examples

[077] Preparation of nylon stock solution for electrospinning

[078] Example 1. provides an exemplary procedure for preparing a nylon solution for electrospinning in accordance with certain embodiments of this invention.

[079] Nylon 6 was supplied by BASF Corp., Florham Park, NJ, USA, under the trademark Ultramid B24. Solutions of the Nylon 6 were prepared in a mixture of two solvents: acetic acid and formic acid, present in weight ratio 2:1. To generate fibers of approximately 120 nm in diameter, an 11% wt. solution of Nylon 6 was prepared, to generate fibers of about 160 nm in diameter a 12.4% wt. solution of Nylon 6 was prepared, and to generate fibers of about 200 nm in diameter, a 13.7% wt. solution was prepared. The solutions were prepared by vigorously stirring each mixture of solvents and polymer in a glass reactor for 5 to 6 hours at 80 °C. The solutions were subsequently cooled to room temperature.

[080] In Example 1, nylon nanofiber mats are made from nanofibers having two different fiber diameter sizes produced by electrospinning, wherein (1) the first mat is approximately 20 µm thick and contains nanofibers having a fiber diameter approximately 120 nm, and designed to retain the bacteria *b. diminuta*, and (2) the second mat is also approximately 20 µm thick, and contains nanofibers having a fiber diameter approximately 200 nm, wherein the second mat has a proportionally higher pore size than the first mat.

[081] The second mat can function as a prefilter layer for the 120 nm diameter fiber containing first mat.

[082] One set of filtration devices were prepared such that the 200 nm fiber diameter containing second mat was layered directly over the 120 nm diameter fiber containing first mat, in an OptiScale 25 capsule format (EMD Millipore Corporation, Billerica, MA). OptiScale 25 capsule devices contain membrane discs having a nominal 25 mm diameter and contain 3.5 cm^2 of frontal surface area.

[083] A second set of filtration devices were prepared such that a polypropylene nonwoven interlayer having fiber diameter in the range of 5 µm to 10 µm was inserted in between the 200 nm diameter fiber containing second mat and 120 nm diameter fiber containing first mat.

[084] Each of these filter arrangements were then challenged with a stream consisting of 2 g/l EMD soy (cell culture media) in water filtered through a 0.45 µm rated membrane, operated at 2 psig.

[085] Figure 2 illustrates that a filter arrangement having a porous second mat containing nanofibers having a fiber diameter of approximately 200 nm layered directly on a porous first mat containing nanofibers having a fiber diameter of approximately 120 nm achieved a higher throughput capacity when compared to a filter arrangement having a single porous mat containing nanofibers having a fiber diameter of approximately 120 nm. However, it was surprisingly discovered that a filtration structure containing a coarse nonwoven interlayer located between the porous nanofiber mat containing fibers having diameter of approximately 200 nm and the porous nanofiber mat containing fibers having a diameter of approximately 120 nm exhibited a throughput capacity higher than either one of the first two filter arrangements.

[086] Example 2 further demonstrates the advantage of including a coarse porous interlayer between two filter layers where, in addition to the mats containing fibers having diameters of approximately 120 nm and 200 nm of Example 1, an additional nanofiber containing mat was generated approximately 20 microns thick containing fibers having a diameter of approximately 160 nm. This additional nanofiber containing mat was placed in between the 120 nm fiber containing mat and 200 nm fiber containing mat.

[087] Figure 2 illustrates a first set of filtration devices wherein the nanofiber containing mats were layered directly over each other. For the second set of filtration devices, a coarse polypropylene nonwoven layer having an approximate fiber diameter of about 5 µm to 10 µm

was placed in between the 120 nm fiber containing mat and 160 nm fiber containing mat, and the 160 nm fiber containing mat and 200 nm fiber containing mat. As in Example 1, all the filter structures were placed into OptiScale 25 capsules.

[088] As in Example 1, the graded fiber structures outperformed the single layer structures. Furthermore, the structures containing the nonwoven coarse interlayers outperformed the structures where the nanofiber mats were layered directly against each other.

[089] Asymmetric or graded pore structure filters allow for improved throughput capacity compared to symmetric structures. It was surprising that adding a porous support in between nanofiber layers of different fiber sizes further enhances the capacity benefit of the asymmetric structure.

[090] Example 3 also demonstrates the advantage of including a coarse porous interlayer in between two filtration layers. The three nanofiber layers described in Example 2 are arranged such that a nylon nonwoven interlayer about 75 µm thick and consisting of fibers having a fiber diameter of about 15 µm to 20 µm is placed adjacent to the 160 nm nanofber layer. In another variant, the nylon nonwoven interlayer is placed only between the 160 nm and 120 nm nanofiber layers, and the 200 nm nanofiber layer was placed adjacent to the 160 nm nanofiber layer.

[091] The throughput capacity of these two filtration structures were measured as described in Example 1, and compared to the filtration structure where no nonwoven interlayer was present (i.e., the three nanofiber layers are adjacent to each other), and the filtration structure containing, and placed between the 160 nm and 120 nm nanofiber layers.

[092] Figure 4 illustrates that the highest performing composite media filter structure had a nonwoven interlayer placed between both the 200 nm and 160 nm nanofiber containing layer and the 160 nm and 120 nm nanofiber containing layer. The composite media filter structure having the nonwoven interlayer placed only between the 200 nm and 160 nm nanofiber containing layers exhibited a capacity performance intermediate to the filter structure without any nonwoven layers and the structure with two nonwoven layers. The dirt holding capacity performance of the filter structure where the nylon nonwoven interlayer was placed only between the 160 nm and 120 nm nanofiber containing layers was not significantly different than the filter structure without any nonwoven interlayer.

[093] Example 4 demonstrates the advantage of including a coarse porous interlayer in between two filtration layers that are designed for retention of mycoplasma and retroviruses.

Nanofiber mats consisting of 70 nm nanofibers have pore sizes capable of retaining mycoplasma, and nanofiber mats consisting of 40 nm fibers have pore sizes capable of retaining retroviruses. Nanofiber mats of 70 nm and 40 nm were joined to the three nanofiber layer structure described in example 3. In one variant, a nylon nonwoven interlayer about 75 μm thick and consisting of fibers having a fiber diameter of about 15 μm to 20 μm was placed between the 70 nm and 40 nm nanofiber layers. In a second variant the 70 nm and 40 nm fiber layers did not have an interlayer between them.

[094] The throughput capacity of these two filtration structures were measured as described in Example 1. Figure 5 illustrates that the composite media filter structure containing a nonwoven interlayer placed between both the 70 nm and 40 nm nanofiber containing layer exhibited a capacity performance superior to the filter structure without any nonwoven layers between the 70 and 40 nm nanofiber layers.

[095] Methods of use

[096] In certain embodiments the invention may be used for filtering, separating, preparing, identifying, detecting, and/or purifying liquid biopharmaceutical preparation samples of interest from liquid samples.

[097] In other embodiments, the present invention can be used with sample preparation methods including, but not limited to, chromatography; high pressure liquid chromatography (HPLC); electrophoresis; gel filtration; sample centrifugation; on-line sample preparation; diagnostic kits testing; diagnostic testing; transport of chemicals; transport of biomolecules; high throughput screening; affinity binding assays; purification of a liquid sample; size-based separation of the components of the fluid sample; physical properties based separation of the components of the fluid sample; chemical properties based separation of the components of the fluid sample; biological properties based separation of the components of the fluid sample; electrostatic properties based separation of the components of the fluid sample; and, combinations thereof.

[098] Conventional processes for protein production often involve cell culture methods in which mammalian or bacterial cell lines are recombinantly engineered to produce the protein of interest. Filters can be used to reduce bioreactor contamination by microorganisms such as bacteria, fungi, mycoplasma, and viruses. For example, nanofiber membranes that include a mat of nanofibers of 120 nm in diameter are suitable for the removal of bacteria of genus

Brevendumonas diminuta. Nanofiber membranes that include a mat of nanofibers of 70 nm in diameter are suitable of the removal of mycoplasma, principally of the genus *Acholeplasma laidlawii*.

[099] The growth of cells in bioreactors is aided by the use of cell culture media, which contain pools of proteins, amino acids, and additives. These additives can clog the filter as they become trapped in the filter medium, closing fluid pathways, and thereby limiting the life of the filter. As described in example 1, EMD soy is a cell culture media that can clog the bacterial retentive layer in a nanofiber filter. Therefore, while filters that remove bacteria or mycoplasma can be used to filter the media, they may have a limited life due to the described clogging phenomenon. A filter employing the embodiments of this invention, in which a prefilter layer and a nonwoven interlayer is placed upstream of the bacterially retentive nanofiber layer for example, can be used to filter this media and will have a longer life compared to filters made using the prior art.

[0100] 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.

[0101] Preferred embodiments of this invention are described herein, include the best mode known to the inventors for carrying out the invention. Of course variations of the preferred embodiments will 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 other 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. The disclosure set forth above may encompass multiple distinct inventions with independent utility. Although each of these inventions has been disclosed in its preferred form(s), the specific embodiments thereof as disclosed and illustrated herein are not to be considered in a limiting sense, because numerous variations are possible.

<u>The present invention encompasses the following embodiments:</u>

(1) A composite media comprising:

a porous interlayer having opposing first and second sides,

a first fiber containing filter layer located on the first side of the interlayer; and

a second fiber containing filter layer located on the second side of the interlayer, wherein the fibers in the first and second fiber containing filter layers have a different fiber diameter, and each fiber containing filter layer has a different pore size rating, and the interlayer has a coarser pore size than either the first or second fiber containing filter layers.

(2) The media according to (1), wherein the fibers in the first and second fiber containing filter layers are a polymer material selected from the group consisting of thermoplastic polymers and thermoset polymers.

(3) The media according to (2), wherein the fibers are nanofibers.

(4) The media according to (1), wherein the interlayer comprises a nonwoven.

(5) The media according to (4), wherein the nonwoven comprises a polymeric nanofiber.

(6) The media according to (5), wherein the polymeric nanofiber is made from polypropylene.

(7) The media according to (3), wherein the nanofibers in the first fiber containing filter layer have a fiber diameter from about 10 nm to about 1,000 nm, and the nanofibers in the second fiber containing filter layer have a fiber diameter from about 10 nm to about 1,000 nm.

(8) The media according to (1), wherein the first and second fiber containing filter layers comprise electropsun nanofiber mats.

(9) The media according to (8), wherein the nanofiber is a polymer material selected from the group consisting of thermoplastic polymers and thermoset polymers.

(10) The media according to (8), wherein the nanofiber is a polymer material selected from the group consisting of thermoplastic polymers, thermoset polymers nylon, polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, poly(vinyl chloride), poly(vinyl alcohol), poly(vinyl acetate), poly(vinylidene fluoride), poly(vinyl butylene), copolymers and combinations thereof.

(11) The media according to (8), wherein the nanofiber is a polymer material selected from the group consisting of nylon-6, nylon-6,6, nylon 6,6-6,10, nylon-6 copolymers, nylon-6,6 copolymers, nylon 6,6-6,10 copolymers and mixtures thereof.

(12) A fluid filtration device comprising:

holder having an inlet, a filtrate outlet, a retentate outlet, and the composite media of (1), wherein the composite media is located in the holder and separates the holder into an upstream compartment and a downstream compartment.

(13) The fluid filtration device according to (12) is selected from the group consisting of a plastic holder, laboratory device, syringe, microtiter plate, metal holder, filter cartridge, and cartridge.

WHAT IS CLAIMED IS:

1. A liquid filtration device comprising:

(a) a holder comprising an inlet and a filtrate outlet; and

(b) a composite media located in the holder such that it separates the holder into an upstream compartment and a downstream compartment, the composite media comprising:

(i) a porous interlayer having opposing first and second sides,

(ii) a first fiber containing filter layer located on the first side of the interlayer; and

(iii) a second fiber containing filter layer located on the second side of the interlayer,

wherein the fibers in the first and second fiber containing filter layers have a different fiber diameter, and each fiber containing filter layer has a different pore size, and the interlayer has a coarser pore size than any of the first or second fiber containing filter layers, and wherein the fibers are nanofibers.

2. The liquid filtration device according to claim 1, wherein the fibers in the first and second fiber containing filter layers are a polymer material selected from the group consisting of thermoplastic polymers and thermoset polymers.

3. The liquid filtration device according to claim 1, wherein the interlayer comprises a nonwoven.

4. The liquid filtration device according to claim 3, wherein the nonwoven comprises a polymeric nanofiber.

5. The liquid filtration device according to claim 4, wherein the polymeric nanofiber is made from polypropylene.

6. The liquid filtration device according to claim 1, wherein the nanofibers in the first fiber containing filter layer have a fiber diameter from 10 nm to 1,000 nm, and the nanofibers in the second fiber containing filter layer have a fiber diameter from 10 nm to 1,000 nm.

7. The liquid filtration device according to claim 1, wherein the first and second fiber containing filter layers comprise electrospun nanofiber mats.

8. The liquid filtration device according to claim 7, wherein the nanofiber is a polymer material selected from the group consisting of thermoplastic polymers and thermoset polymers.

9. The liquid filtration device according to claim 7, wherein the nanofiber is a polymer material selected from the group consisting of thermoplastic polymers, thermoset polymers nylon, polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, poly(vinyl chloride), poly(vinyl alcohol), poly(vinyl acetate), poly(vinylidene fluoride), poly(vinyl butylene), copolymers and combinations thereof.

10. The liquid filtration device according to claim 7, wherein the nanofiber is a polymer material selected from the group consisting of nylon-6, nylon-6,6, nylon 6,6-6,10, nylon-6 copolymers, nylon-6,6 copolymers, nylon 6,6-6,10 copolymers and mixtures thereof.

11. A method of removing bacteria from cell culture media comprising filtering the cell culture media through a liquid filtration device according to claim 1.

12. The method of claim 11, wherein the bacteria is removed from the cell culture media.