NEGATIVELY CHARGED MEMBRANE

NEGATIVER GELADENER MEMBRANEN

MEMBRANE CHARGEE NEGATIVEMENT

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Description

[0001] The present invention generally relates to negatively charged membranes, and in particular to negatively charged membranes comprising a porous substrate and a crosslinked coating. The membranes find use in the treatment of fluids containing positively charged species such as proteins, e.g., in ion-exchange chromatography.

BACKGROUND OF THE INVENTION

[0002] Negatively charged ion-exchange membranes have been proposed for the separation and/or purification of biomolecules such as proteins, amino acids, and nucleic acids. For the ion exchange membrane to perform effectively in the above applications, the membrane should satisfy several important parameters. For example, the membrane should exhibit high rates of fluid flow. The membrane should have high dynamic binding capacity for biomolecules, and should be capable of selectively binding the biomolecules, which have different surface charges. The membrane should, therefore, have low non-specific binding, e.g., resulting from hydrophobic interactions. The membrane should withstand high treatment fluid velocities. The preparation of the membrane should not involve chemistries and processes that are cumbersome to practice. Some of the ion exchange membranes known heretofore suffer from the failure to satisfy one or more of the parameters set forth above.

[0003] Accordingly, there exists a need for a cation exchange membrane that exhibits high rates of fluid flow. There further exists a need for a cation exchange membrane that has high dynamic binding capacity and selectivity for biomolecules. There further exists a need for a membrane that has low non-specific binding or low binding that results from hydrophobic interactions. There further exists a need for a membrane that can withstand high fluid flow velocities. There further exists a need for a membrane that involves preparation chemistries and/or processes that are not cumbersome to practice.

[0004] These advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

[0005] U.S. Patent 5,021,160 discloses copolymers synthesized from 2-acrylamido-2-methyl-1-propane sulfonic acid and either N-(isobutoxymethyl) acrylamide or 2-hydroxyethyl methacrylate and a process for preparing anionic charge modified microporous filtration membranes.

[0006] WO 98/17377 discloses charged membranes comprising a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate.

[0007] European Patent Application No. 0 474 617 A1 discloses a surface modified support membrane where-

in the support membrane has a layer of hydrogel deposited on the surface thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Fig. 1 depicts the breakthrough curve for lysozyme obtained on an embodiment membrane of the present invention. The x-axis represents the filtration time, and the y-axis represents the absorbance of the filtrate at 280 nm and is indicative of the concentration of the protein. See Example 2 for additional details.

Fig. 2 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 3 for additional details.

Fig. 3 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 4 for additional details.

BRIEF SUMMARY OF THE INVENTION

[0009] Many of the foregoing needs have been fulfilled by the present invention which provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating. In accordance with the process of the invention as defined in claim 27, the membrane can be prepared from a composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxyalkyl)- and/or N-(alkoxyalkyl)- acrylamides, and a hydrophilic unsaturated monomer.

[0010] The present invention further provides a negatively charged microporous membrane having a protein binding capacity of about 25 mg/ml lysozyme or more comprising a porous substrate and a crosslinked coating that provides a fixed negative charge. The negatively charged microporous membrane of the present invention as defined in claim 1 comprises a porous substrate and a crosslinked coating comprising a polymer having anionic groups and amide-amide and amide-ester crosslinks.

[0011] The membranes of the present invention are advantageously free of covalent bonds or grafts with the substrate.

[0012] The present invention further provides a process for preparing an embodiment of the membrane according to claim 27. The membrane can be optionally washed or leached to remove extractable residue therein.

[0013] The present invention further provides a process as defined by claim 32 for transferring biological material from an electrophoresis gel comprising contacting
the gel with the membrane of the present invention and transferring the biomolecules to the membrane.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0014] The present invention provides embodiments of negatively charged membranes having high charge density, high water flow rates, high dynamic protein binding capacity, and low non-specific protein binding capacity. The membranes of the present invention find use in cation exchange chromatography and in the separation and/or purification of charged species, especially biomolecules such as proteins.

[0015] The present invention provides a negatively charged microporous membrane as defined in claim 1 comprising a porous substrate and a crosslinked coating having anionic groups. The crosslinked coating can be prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxyalkyl)- or N-(alcohol)-acrylamides and a hydrophilic unsaturated monomer. The composition can further include an initiator. In preferred embodiments, the N-(hydroxyalkyl)- or N-(alcohol)-acrylamide is one wherein the alkyl moiety has 4 or less carbon atoms, and more preferably the alkyl moiety is methyl.

[0016] The crosslinked coating may be prepared from a said composition comprising a hydroxyl-rich material such as a polysaccharide, and optionally an initiator. The resulting negatively charged microporous membrane comprises a porous substrate and a crosslinked coating comprising a polymer having anionic groups and amide-amide and amide-ester crosslinks.

[0017] The membrane of the present invention contains fixed anionic groups. The anionic group can be any negatively charged group - sulfonic, carboxylic, phosphonic, and the like, preferably sulfonic or carboxylic acid groups. The coating composition comprises an unsaturated monomer having an anionic group. Any suitable unsaturated monomer - vinyl, vinyl aromatic, acrylic, or other monomer can be used.

[0018] The unsaturated monomer preferably is an acrylic monomer. The acrylic monomer can be an acrylate or an acrylamide. The acrylic monomer is preferably an acrylamide. The term "acrylamide" herein refers to unsubstituted as well as substituted monomers having a -C=C-(C=O)-N- moiety. The nitrogen and the C=C double bond are involved in hydrogen bonding with the hydroxyl and hydroxyalkyl. Thus, preferred hydrophilic acrylic monomers are hydroxyacrylc and hydroxyalkylacrylic. The acrylamide monomer can be an acrylate ester or an acrylamide. An example of a preferred hydroxyl ester acrylamide monomer is hydroxypropyl methacrylate.

[0019] The coating composition further comprises a hydrophilic unsaturated monomer, e.g., a nonionic hydrophilic unsaturated monomer. Any suitable hydrophilic unsaturated monomer can be used, preferably an acrylic monomer. The monomer contains one or more polar groups that contribute hydrophilicity. Examples of such groups include hydroxy, alkoxyl, hydroxyalkyl, and amido. Preferred hydrophilic groups are hydroxyl and hydroxyalkyl. Thus, preferred hydrophilic acrylic monomers are hydroxyacrylc and hydroxyalkylacrylic. The acrylamide monomer can be an acrylate ester or an acrylamide. An example of a preferred hydroxyl acrylate monomer is hydroxypropyl methacrylate.

[0020] The coating composition comprises a crosslinking agent which is at least one or more N-(hydroxyalkyl)- and/or N-(alcohol)-acrylamides. Preferred crosslinking agents include N-(alkoxymethyl)acrylamide and N-(hydroxymethyl)acrylamide. N-(isobutoxymethyl)acrylamide is further preferred.

[0021] The coating composition preferably comprises an initiator. Any suitable initiator - free radical initiator, photoinitiator, and the like, can be used. A free radical initiator is preferred. An example of a suitable free radical initiator is a persulfate such as ammonium persulfate.

[0022] Without being bound to any particular theory, it is believed that the use of the three monomers in certain embodiments contributes to increased spatial separation of charges. Thus, it is believed that the distance between the anionic groups is increased. This increased distance disfavors association of the anionic groups. Accordingly, inter- and/or intra- chain association of anionic groups is reduced compared to a system wherein only an anionic monomer and a crosslinking monomer are employed, particularly in a two monomer system wherein a hydrophilic or hydroxyl-rich material such as a polysaccharide is not employed. The reduced association makes the negatively charged groups available for interaction with positively charged molecules in the treated fluid. This results, for example, in enhanced dynamic protein binding capacity.

[0023] The membrane according to some embodiments is made from a coating composition that includes a hydroxyl-rich material, which may be a small molecule or a polymer having a plurality of hydroxyl groups, e.g., two, three, four or more hydroxyl groups per molecule. Examples of hydroxyl-rich materials include polysaccharides and polyvinyl alcohol, preferably polysaccharides. Without being bound to any particular mechanism, it is believed that the hydroxyl groups of the hydroxyl-rich material involve in hydrogen bonding with the fluid. The saccharide ring repeat units exert steric effects. Operation of one or both of these mechanisms results in increased charge separation among the anionic groups. The increased charge separation is believed to
reduce anion association and facilitate interaction between the anionic sites and the positively charged species in the treated fluid.

[0024] Any suitable polysaccharide can be used, preferably a water soluble polysaccharide. An example of a preferred polysaccharide is dextran. The molecular weight of the dextran is about 40,000,000, e.g., from about 10,000 to about 2,000,000, preferably from about 10,000 to about 500,000, and more preferably from about 10,000 to about 300,000. Particular examples of suitable molecular weights include 110,000 and 148,000.

[0025] The coating composition of certain embodiments can be prepared by combing and polymerizing the acrylic monomer having an anionic group, the non-ionic hydrophilic monomer, the crosslinking agent, and the initiator.

[0026] The polymerization can be carried out in a solvent, preferably in water or water/methanol solution. The polymerization is preferably stopped prior to the formation of a gel or excessive crosslinking. The viscosity of the polymerization solution can be monitored to control the degree of polymerization. The polymerization is carried out for any suitable length of time, e.g., for about 4 hours or more. According to certain embodiments, the polymerization is carried out for a period of about 4 hours to about 5 hours. According to certain other embodiments, the polymerization is carried out for a period of from about 16 hours to about 24 hours. The viscosity of the solution is typically below about 2000 cps (2 Pa.s), e.g., preferably from about 50 cps (0.05 Pa.s) to about 500 cps (0.5 Pa.s), and more preferably from about 100 cps (0.1 Pa.s) to about 500 cps (0.5 Pa.s). According to certain embodiments, the viscosity is from about 100 cps (0.1 Pa.s) to about 250 cps (0.25 Pa.s).

[0027] The polymerization solution can contain the anionic acrylic monomer (A), the crosslinking agent (B), and the non-ionic hydrophilic monomer (C) in a suitable ratio. The percentage of each monomer (A, B, or C) can be from about 0.1 to 30% by weight, preferably from about 0.1 to 20% by weight.

[0028] The crosslinked coating comprises amide-ester crosslinks that form as a result of the reaction of the nonionic hydrophilic monomer with the crosslinking agent. For example, these bonds form as a result of the reaction of the hydroxyl groups in the hydroxyalkyl acrylate with the N-((isobutoxymethyl)-acrylamide. In addition, amide-amide crosslinks also form as a result of the reaction between the nonionic hydrophilic monomers. For example, the amide-ester crosslink can have the formula:

\[-C(\ce{=O})O-R-NH-C(\ce{=O}),\]

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably \(-\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-}\). The amide-amide crosslink can have the formula:

\[-\text{C(=O)NH-R-NH-C(=O)},\]

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably \(-\text{CH}_2\text{-O-CH}_2\text{-}\).

[0029] The coating solution contains the anionic polymer prepared as above and, optionally, a polysaccharide, preferably a dextran. The anionic polymer and the polysaccharide can be present in the coating solution in the ratio of from about 100:1 to about 1:100, preferably from about 10:1 to about 1:10, and more preferably from about 5:1 to about 1:5.

[0030] The coating solution contains the anionic polymer and, optionally dextran, in an amount of from about 0.01% to about 15% by weight, preferably from about 0.1% to about 10% by weight, and more preferably from about 0.5% to about 5% by weight of the coating solution. For example, the coating solution can contain 4.5% by weight of polymer and 1.5% by weight of dextran.

[0031] The pH of the coating solution can be adjusted suitably. For example, the pH of the coating solution containing a carboxylated polymer can be adjusted to about 3.0 to about 4.0 and preferably about 3.75. The pH of the coating can be adjusted by the addition of an acid or base. An example of a suitable base is 2N NaOH aqueous solution.

[0032] The coating solution is coated on a porous substrate, preferably a hydrophilic substrate. The hydrophilic porous substrate can be made of any suitable material; preferably, the substrate comprises a polymer. Examples of suitable polymers include polyaromatics, polysulfones, polylefins, polystyrenes, polycarbonates, polyamides, polyimides, fluoropolymers, cellulotic polymers such as cellulose acetates and cellulose nitrate, and PEEK. Aromatic polysulfones are preferred. Examples of aromatic polysulfones include polyethersulfone, bisphenol A polysulfone, and polyphenylsulfone. Polyethersulfone is particularly preferred. The porous substrate can have any suitable pore size, for example, a pore size of below about 10 µm, e.g., from about 0.01 µm to about 10 µm, preferably from about 0.1 µm to about 5 µm, and more preferably from about 0.2 µm to about 5 µm. The porous substrate can be asymmetric or, in a preferred embodiment, symmetrical.

[0033] The porous substrate can be prepared by methods known to those of ordinary skill in the art. For example, the porous substrate can be prepared by a phase inversion process. Thus, a casting solution containing the polymer, a solvent, a pore former, a wetting agent, and optionally a small quantity of a non-solvent is prepared by combining and mixing the ingredients, preferably at an elevated temperature. The resulting solution is filtered to remove any impurities. The casting solution is cast or extruded in the form of a sheet or hollow fibers. The resulting sheet or fiber is allowed to set or gel as a phase inverted membrane. The set membrane is then leached to remove the solvent and other soluble
[0034] The porous substrate can be coated with the coating solution by methods known to those of ordinary skill in the art, for example, by dip coating, spray coating, meniscus coating, and the like. Dip coating, for example, can be carried out as follows. The substrate is immersed in the solution for a given period of time sufficient to insure complete or substantially complete coating of the pore walls. The immersion time can be from about 1 second to 1.0 minute, preferably from about 0.1 minutes to about 0.5 minutes, and more preferably from about 1/6 minute to about 1/3 minute. Following the immersion, the excess coating solution on the substrate is removed by allowing it to drain under gravity or by the use of a squeegee or air knife. The resulting coated substrate is cured to effect the curing or crosslinking of the coating.

[0035] Thus, the membrane can be cured below 150°C, e.g., at a temperature of from about 60°C to about 130°C, and preferably at a temperature of from about 80°C to about 130°C, for a suitable period of time, which can range from about 5 minutes to about 120 minutes and preferably from about 5 minutes to about 60 minutes. According to certain embodiments, the membrane is cured at a temperature of from about 120°C to about 125°C for a period of from about 20 minutes to about 30 minutes.

[0036] The resulting membrane can be washed to leach out any extractable in the membrane. Certain embodiments of the membrane, particularly a membrane having carboxyl functionality, are washed or leached in a basic solution, preferably at a pH of from about 8 to about 12. The leaching liquid can be prepared by adding a base such as sodium hydroxide, sodium carbonate, or sodium bicarbonate. The base can be added as a solid or a solution. Particular examples of pHs of the leaching liquid are about 11.9, about 11.4, and about 8.1. These pHs can be achieved by the use of, e.g., a 2N NaOH solution, sodium carbonate, or sodium bicarbonate.

[0037] Illustratively, a carboxylated membrane can be washed or leached at a temperature of from about 37°C to about 93°C or higher and preferably from about 54°C to about 73°C or higher. A sulfonic acid containing membrane can be washed or leached at a temperature of from about 54°C to about 93°C or higher. If desired, the device can include a plurality of membranes, e.g., to provide a multilayered filter element, or stacked to provide a membrane module, such as a membrane module for use in membrane chromatography. Filter cartridges can be constructed by including a housing and endcaps to provide fluid seal as well as at least one inlet and at least one outlet. The devices can be constructed to operate in crossflow or tangential flow mode as well as dead-end mode. Accordingly, the fluid to be treated can be passed, for example, tangentially to the membrane surface, or passed perpendicular to the membrane surface.

[0038] The present invention provides a process as defined in claim 27 for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having pendant anionic groups. An embodiment of the process comprises:

(a) providing a porous substrate;
(b) contacting the substrate with a hydroxyl-rich material and a composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxyalkyl)- and/or N-(alkoxyalkyl)- acrylamides, a hydrophilic unsaturated monomer, and optionally an initiator;
(c) curing the substrate obtained in (b) to obtain the negatively charged membrane; and
(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

[0039] In an embodiment of the present invention the crosslinked coating is prepared from a composition comprising an unsaturated monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)-acrylamide, a nonionic hydrophilic acrylic monomer, and an initiator.

[0040] In a further embodiment of the present invention the crosslinked coating is prepared from a composition comprising an acrylic monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)- acrylamide, a nonionic hydrophilic acrylic monomer, and an initiator.

[0041] The membrane of the present invention may be incorporated in a device, e.g., a filter device, chromatography device, macromolecular transfer device, flow distributor arrangement, and/or a membrane module which may comprise one or more negatively charged membranes of the present invention. The device can be in any suitable form. For example, the device can include a filter element comprising the negatively charged membrane in a substantially planar or pleated form. The element can have a hollow generally cylindrical form. If desired, the device can include the filter element in combination with upstream and/or downstream support or drainage layers. The device can include a plurality of membranes, e.g., to provide a multilayered filter element, or stacked to provide a membrane module, such as a membrane module for use in membrane chromatography. Filter cartridges can be constructed by including a housing and endcaps to provide fluid seal as well as at least one inlet and at least one outlet. The devices can be constructed to operate in crossflow or tangential flow mode as well as dead-end mode. Accordingly, the fluid to be treated can be passed, for example, tangentially to the membrane surface, or passed perpendicular to the membrane surface.

[0042] For embodiments of the membrane which are in the form of a tube or fiber, or bundles of tubes or fibers, the membrane can be configured as modules, e.g., after potting their ends with an adhesive. For a description of illustrative chromatographic devices, porous medium modules, and flow distributor arrangements, see U.S. Provisional Patent Application Nos. 60/121,667 and 60/121,701, both filed on February 25, 1999; U.S. Pro-
and preferably above 10 mL/min/cm², e.g., from about 10 mL to about 1000 mL, and charge density. Thus, for example, the membrane preferably has a water flow rate above 5 mL/min/cm², and preferably above 10 mL/min/cm², e.g., from about 20 mL/min/cm² to about 160 mL/min/cm², and preferably from about 25 mL/min/cm² to about 100 mL/min/cm² at 24 inch Hg. The membrane is robust and can withstand high treatment fluid flow rates. Thus, the membrane can be subjected to flow rates up to 10 cm/min, for example, from about 1 cm/min to 10 cm/min at 10 psi (68.9 kPa). The membrane has an open water bubble point of below about 70 psi (482 kPa), e.g., from about 2.5 psi (9.39 kPa) to about 70 psi (482 kPa), and preferably from about 5 psi (34.47 kPa) to about 50 psi (344.7 kPa).

[0043] The membrane of the present invention has a high charge density. The charge density of the membrane can be measured by methods known to those of ordinary skill in the art. For example, the charge density can be measured by the membrane’s ability to bind a positively charged dye. Illustratively, the membrane has a Methylene Blue dye binding capacity of at least about 10 mL, e.g., from about 10 mL to about 1000 mL, and preferably from about 100 mL to about 800 mL when tested with a 10 ppm dye solution in water. Methylene Blue is a positively charged dye. The dye binding capacity is measured by filtering under a 24 inch Hg negative pressure, a 10 ppm by weight solution, pH 6.6, of Methylene Blue dye in a membrane disc of 25 mm diameter, and monitoring the volume of the filtrate until a trace of the dye begins to appear in the filtrate.

[0045] The membrane of the present invention has a high specific protein binding capacity. The membrane has a lysozyme specific binding capacity of above 10 mg/mL, e.g., from about 10 mg/mL to about 130 mg/mL and preferably from about 25 mg/mL to about 120 mg/mL. The specific binding capacity can be determined by the following illustrative method. A fluid containing a lysozyme protein in 10 mM MES buffer, pH 5.5, is filtered by passing through a membrane at 1 cm/min and the concentration of the protein in the filtrate is measured as a function of time. The concentration of the protein can be determined spectrophotometrically, e.g., by measuring the absorbance of the protein at 280 nm. A breakthrough curve such as the one shown in Fig. 1 can then be constructed with the x-axis depicting the time of the filtration experiment and the y-axis depicting the protein concentration in the filtrate. The membrane has high specific protein binding capacity and low non-specific or hydrophobic binding. The slope of the breakthrough curve obtained on the membrane is vertical or substantially vertical. This characteristic offers improved resolution and separation of proteins. The membrane also has high dynamic protein binding capacity.

[0046] An advantage of the membrane of the present invention is that proteins do not leak prior to breakthrough. Another advantage of the present invention is that the components of the membrane are carefully chosen so that the membrane is free or substantially free of grafts or covalent links between the coating and the substrate. The preparation of negatively charged membranes of the present invention involves a chemistry and procedure that is relatively simple and easy to practice.

[0047] The properties of the membranes of the present invention make them attractive for use in the detection, separation, and/or purification of biomolecules such as proteins, amino acids, nucleic acids, and viruses. Examples of nucleic acids include modified or unmodified, synthetic or natural RNA and DNA.

[0048] The membranes of the present invention find use in various applications such as filtration of fluids containing positively charged atoms, molecules, and particulates, and macromolecular transfer from electrophoresis gels such as the transfer of nucleic acids and proteins from electrophoresis gels to an immobilizing matrix. The membrane can find use in the separation or purification of components present in biological fluids. Thus, for example, the membrane can find use in the purification of human albumins from the serum, in the therapeutic fractionation of blood, and separation of the components in genetically engineered cell cultures or fermentation broths. The membrane can be used in the purification of, for example, viral vaccines and gene therapy vectors such as adeno-associated viruses.

[0049] Accordingly, the present invention provides a process for treating a fluid containing biomolecules according to claim 33. The positively charged materials adsorbed on the membrane can be recovered by eluting with a suitable solvent eluant. The present invention further provides a process for selectively adsorbing one or more biomolecules from fluid containing a mixture of biomolecules comprising contacting the fluid with the membrane under conditions favorable to the adsorption of selected biomolecules. The one or more biomolecules may be selectively released from a membrane of the present invention by way of contacting the membrane having adsorbed biomolecules with an eluant under conditions favorable to the release of the selected biomolecules. The present invention further provides a process for macromolecular transfer from an electrophoresis gel comprising contacting a membrane of the...
present invention with the electrophoresis gel, and transferring the macromolecules from the gel to the membrane.

[0050] The negatively charged membrane of the present invention is particularly suitable for treating fluids containing biomolecules that have a positive surface charge for the given pH of the fluid. For example, lysozyme has an isoelectric point of 11.25, and it can be purified by using the negatively charged membrane of the present invention from a low salinity, for example 10 mM MES, fluid that is pH 5.5. Proteins with different surface charges may also be separated using the membrane of the present invention, for example separating lysozyme from Cytochrome C.

[0051] Thus, a mixture of lysozyme and Cytochrome C can be separated as follows. 80 µl of a fluid containing 3 mg/ml lysozyme and 1 Cytochrome C can be placed on a chromatographic column or stack of 5 layers of a 25 mm diameter negatively charged membrane of the present invention. The column or stack can be eluted of 5.5 to 1M NaCl-10 mM MES buffer at a pH of 5.5. The flow rate can be 4 ml/min. Cytochrome C elutes first, followed by lysozyme.

[0052] The following examples further illustrate the present invention.

EXAMPLE 1

[0053] This Example illustrates a method of preparing a polymer composition for preparing an embodiment of the negatively charged membrane of the present invention.

[0054] 2-Acrylamido-2-methyl-1-propanesulfonic acid, N-(isobutoxymethyl)acrylamide, and hydroxypropyl methacrylate were combined in a weight ratio of 8.0:2.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 12% by weight. Ammonium persulfate was used as the initiator at 0.3% by weight of the solution. The polymerization was carried out for a period of about 10-15 at ambient temperature (24-25°C). The resulting solution had a viscosity of 166 cps (0.166 Pa.s).

EXAMPLE 2

[0055] This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

[0056] A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 4:1.

[0057] A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain an embodiment of the present invention.

[0058] The membrane obtained above was tested for treatment of a solution containing lysozyme. The solution was contained 206.4 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 1. The membrane had a lysozyme binding capacity of 97 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

EXAMPLE 3

[0059] This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

[0060] A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 4:1.

[0061] A hydrophilic microporous cellulose nitrate substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain an embodiment of the present invention.

[0062] The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 201.3 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 2. The membrane had a lysozyme binding capacity of 77 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

EXAMPLE 4

[0063] This Example illustrates a method for preparing another embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

[0064] 2-Acrylamidoglycolic acid, 2-carboxethyl acrylate, N-(isobutoxymethyl)acrylamide, N-(hydroxymethyl)-acrylamide, and hydroxypropyl acrylate were combined in a weight ratio of 5.0:5.0:3.0:1.5:1.5 in a meth-
anol-water medium to obtain a polymerization solution having a solids content of 16% by weight. Ammonium persulfate was used as the initiator at 0.4% by weight of the solution. The polymerization was carried out for a period of about 16-24 hours at ambient temperature. The resulting solution had a viscosity of 116 cps (0.116 Pa.s). A coating solution was prepared by mixing the polymerization solution and a water solution of dextran, molecular weight 148 K, so that the resulting solution contained 4% polymer and 1.33% dextran by weight.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm coated with the above coating solution. The coated substrate was cured in an oven at 100-110 °C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain another embodiment of the present invention.

The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 213.6 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 3. The membrane had a lysozyme binding capacity of 45 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

Claims

1. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating, wherein the crosslinked coating is obtainable by polymerizing a mixture that comprises an unsaturated monomer comprising a negatively charged group, a hydrophilic non-ionic unsaturated monomer, and a N-(hydroxyalkyl) or N-(alkoxyalkyl)-acrylamide monomer, wherein the crosslinked coating comprises amide-ester and amide-amide crosslinks.

2. The negatively charged microporous membrane of claim 1, wherein the hydrophilic non-ionic unsaturated monomer is an acrylic monomer.

3. The negatively charged microporous membrane of claim 1 or claim 2, wherein the N-(hydroxyalkyl)- or N-(alkoxyalkyl)-acrylamide monomer includes an alkyl group of 4 carbon atoms or less.

4. The negatively charged microporous membrane of any of claims 1-3, wherein the crosslinked coating includes a hydroxyl-rich material comprising the crosslinked coating is such that the ratio of the polymer to the hydroxyl-rich material is between 100:1 and 1:100.

5. The negatively charged microporous membrane of claim 4, wherein the hydroxyl-rich material is a polysaccharide.

6. The negatively charged microporous membrane of any of claims 1-5, wherein the negatively charged group is a sulfonic or carboxylic acid group.

7. The negatively charged microporous membrane of claim 2, wherein the acrylic monomer is an acrylate or acrylamide.

8. The negatively charged microporous membrane of claim 7, wherein the acrylic monomer is an acrylamide.

9. The negatively charged microporous membrane of claim 8, wherein the acrylamide is an alkylacrylamide.

10. The negatively charged microporous membrane of claim 9, wherein the acrylamide comprises a sulfonic acid group.

11. The negatively charged microporous membrane of claim 10, wherein the acrylamide is acrylamido-N-alkylsulfonic acid.

12. The negatively charged microporous membrane of claim 9, wherein the alkylacrylamide comprises a carboxylic acid group.

13. The negatively charged microporous membrane of claim 12, wherein the mixture includes a further acrylic monomer comprising a carboxylic acid group.

14. The negatively charged microporous membrane of claim 13, wherein the further acrylic monomer is an acrylate.

15. The negatively charged microporous membrane of claim 14, wherein the acrylate is β-carboxyethyl acrylate.

16. The negatively charged microporous membrane of claim 4, wherein the acrylic monomer is a hydroxyacrylic monomer.

17. The negatively charged microporous membrane of claim 16, wherein the hydroxyacrylic monomer is a hydroxyacrylamide or a hydroxyacrylate.

18. The negatively charged microporous membrane of
any of claims 1-17, wherein the mixture includes an N-(alkoxymethyl) acrylamide.

19. The negatively charged microporous membrane of claim 18, wherein the N-(alkoxymethyl) acrylamide is N-isobutoxymethyl acrylamide.

20. The negatively charged microporous membrane of claim 5, wherein the polysaccharide is dextran.

21. The negatively charged microporous membrane of any of claims 1-20, wherein the membrane has a dynamic protein binding capacity of about 25 mg/ml lysozyme or more.

22. The negatively charged microporous membrane of any one of claims 1-21, wherein the material forming the substrate comprises a polymer selected from the group consisting of polyaromatics, polysulfones, polylefins, polystyrenes, polyamides, polymides, cellulose acetates, cellulose nitrates, polycarbonates, polyesters, and fluoropolymers.

23. The negatively charged microporous membrane of claim 22, wherein the substrate comprises a polysulfone.

24. The negatively charged microporous membrane of any of claims 1-23, wherein the porous substrate is hydrophilic.

25. The negatively charged microporous membrane of any one of claims 1-24, wherein an unsaturated unit comprising a negatively charged group forms 0.1% to 20% w/w of the crosslinked coating.

26. The negatively charged microporous membrane of any one of claims 1-25, wherein 0.1% to 15% w/w of the crosslinked coating is composed of the polymer or 0.1% to 15% w/w of the crosslinked coating is composed of the polymer and dextran.

27. A process for preparing a negatively charged microporous membrane of claim 1 comprising a porous substrate and a crosslinked coating including negatively charged groups comprising:
   (a) providing a porous substrate;
   (b) forming a mixture comprising an unsaturated, monomer that comprises a negatively charged group, a hydrophilic non-ionic unsaturated monomer, and a N-(hydroxyalkyl)- or N-(alkoxyalkyl)-acrylamide monomer and polymerizing the mixture to form a polymer; 
   (c) contacting the substrate with the polymer; and
   (d) curing the substrate obtained in (c) to obtain the negatively charged microporous membrane comprising a crosslinked coating including negatively charged groups.

28. The process of claim 27, wherein the negatively charged group is a sulfonic or carboxylic acid group.

29. The process of claim 27 or claim 28, wherein the unsaturated monomer that comprises a negatively charged group is an acrylic monomer comprising a sulfonic or carboxylic acid group.

30. The process of claim 29, wherein the acrylic monomer comprising a sulfonic or carboxylic acid group is an acrylate or an acrylamide.

31. The process of claim 27, wherein the process includes contacting the substrate with a hydroxyl-rich material comprising two or more hydroxyl groups per molecule before the substrate is cured so that the amount of the hydroxyl-rich material in the crosslinked coating is such that the ratio of the polymer to the hydroxyl-rich material in the membrane is between 100:1 and 1:100.

32. A process for transferring biological material from an electrophoresis gel comprising contacting the electrophoresis gel with the membrane of any of claims 1-26 and transferring a protein, a polypeptide, an amino acid, a nucleic acid, or a combination of any thereof from the gel to the membrane.

33. A process for separating positively charged biomolecules from a fluid, the biomolecules being selected from the group consisting of proteins, amino acids, nucleic acids and viruses, said process comprising placing said fluid in contact with the negatively charged microporous membrane of any one of claims 1 to 26 so as to adsorb the positively charged biomolecules to said membrane.

Patentansprüche


2. Negativ geladene mikroporöse Membran nach Anspruch 1, wobei das hydrophile nichtionische ungesättigte Monomer ein Acryl-Monomer ist.

4. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 3, wobei die vernetzte Beschichtung ein hydroxylreiches Material mit zwei oder mehr Hydroxylgruppen pro Molekül oder Einheit umfasst, wobei die Menge des hydroxylreichen Materials in der vernetzten Beschichtung so ist, dass das Verhältnis des Polymers zu dem hydroxylreichen Material in einem Bereich von 100:1 bis 1:100 angesiedelt ist.

5. Negativ geladene mikroporöse Membran nach Anspruch 4, wobei das Acryl-Monomer ein Acrylat ist.

6. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 5, wobei die negativ geladene Gruppe eine Sulfon- oder Carbonsäure-Gruppe ist.

7. Negativ geladene mikroporöse Membran nach Anspruch 2, wobei das Acryl-Monomer ein Acrylamid ist.


10. Negativ geladene mikroporöse Membran nach Anspruch 9, wobei das Acrylamid eine Sulfonsäure-Gruppe enthält.


12. Negativ geladene mikroporöse Membran nach Anspruch 9, wobei das Alkylacrylamid eine Carbonsäure-Gruppe enthält.


15. Negativ geladene mikroporöse Membran nach Anspruch 14, wobei das Acrylat β-Carboxyethylacrylat ist.


17. Negativ geladene mikroporöse Membran nach Anspruch 16, wobei das Hydroxyacryl-Monomer ein Hydroxyacrylamid oder ein Hydroxyacrylat ist.

18. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 17, wobei die Mischung ein N-(Alkoxymethyl)-acrylamid umfasst.


21. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 20, wobei die Membran ein dynamisches Protein-Bindevermögen von ca. 25 mg/ml Lysozym oder mehr aufweist.

22. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 21, wobei das Substrat bildende Material ein Polymer umfasst, ausgewählt aus der Gruppe, welche aus Polyaromaten, Polysulfonen, Polyolefinen, Polystyrolen, Polyamiden, Polyimiden, Celluloseacetaten, Cellulosinitrat en, Polycarbonaten, Polyestern und Fluoropolymeren besteht.

23. Negativ geladene mikroporöse Membran nach Anspruch 22, wobei das Substrat ein Polysulfon umfasst.

24. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 23, wobei das poröse Substrat hydrophil ist.

25. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 24, wobei eine ungesättigte Einheit, welche eine negativ geladene Gruppe umfasst, 0,1 % bis 20 % w/w der vernetzten Beschichtung bildet.

26. Negativ geladene mikroporöse Membran nach einem der Ansprüche 1 bis 25, wobei 0,1 % bis 15 % w/w der vernetzten Beschichtung aus dem Polymer bestehen oder 0,1 % bis 15 % w/w der vernetzten Beschichtung aus dem Polymer und Dextran bestehen.
27. Verfahren zum Herstellen einer negativ geladenen mikroporösen Membran nach Anspruch 1, umfassend ein poröses Substrat und eine vernetzte Beschichtung mit negativ geladenen Gruppen, umfassend:

(a) Bereitstellen eines porösen Substrates;

(b) Bilden einer Mischung, umfassend ein ungesättigtes Monomer mit einer negativ geladenen Gruppe, ein hydrophiles nichtionisches ungesättigtes Monomer und ein N-(Hydroxyalkyl)- oder N-(Alkoxyalkyl)-acrylamid-Monomer, und Polymerisieren der Mischung, um ein Polymer zu bilden;

(c) Kontaktieren des Substrates mit dem Polymer; und

(d) härten des in (c) erhaltenen Substrates, um die negativ geladene mikroporöse Membran zu erhalten, welche eine vernetzte Beschichtung mit negativ geladenen Gruppen umfasst.

28. Verfahren nach Anspruch 27, wobei die negativ geladene Gruppe eine Sulfon- oder Carbonsäure-Gruppe ist.

29. Verfahren nach Anspruch 27 oder Anspruch 28, wobei das ungesättigte Monomer, welches eine negativ geladene Gruppe aufweist, ein Acrylmonomer ist, welches eine Sulfon- oder Carbonsäure-Gruppe enthält.

30. Verfahren nach Anspruch 29, wobei das Acryl-Monomer, welches eine Sulfon- oder Carbonsäure-Gruppe aufweist, ein Acrylat oder ein Acrylamid ist.

31. Verfahren nach Anspruch 27, wobei das Verfahren umfasst: Kontaktieren des Substrates mit einem hydroxyrichen Material, welches zwei oder mehr Hydroxygruppen pro Molekül enthält, vor dem Härten des Substrates, so dass die Menge des hydroxylichen Materials in der vernetzten Beschichtung so ist, dass das Verhältnis des Polymers zu dem hydroxylichen Material in der Membran in einem Bereich von 100:1 bis 1:100 angesiedelt ist.


33. Verfahren zum Abtrennen positiv geladener Biomoleküle von einem Fluid, wobei die Biomoleküle ausgewählt sind aus der Gruppe, welche aus Proteinen, Aminosäuren, Nukleinsäuren und Viren besteht, wobei das Verfahren umfasst: Inkontaktkbringen des Fluids mit der negativ geladenen mikroporösen Membran nach einem der Ansprüche 1 bis 26, um die positiv geladenen Biomoleküle an der Membran zu adsorbieren.

**Revendications**

1. Membrane microporeuse chargée négativement comprenant un substrat poreux et un revêtement réticulé, dans laquelle le revêtement réticulé peut être obtenu en polymérisant un mélange qui comprend un monomère insaturé comprenant un groupe chargé négativement, un monomère insaturé non ionique hydrophile et un monomère N-(hydroxyalkyl)- ou N-(alcoxyalkyl)-acrylamide, dans laquelle le revêtement réticulé comprend des liaisons transversales amide-ester et amide-amide.

2. Membrane microporeuse chargée négativement de la revendication 1, dans laquelle le monomère insaturé non ionique hydrophile est un monomère acrylique.

3. Membrane microporeuse chargée négativement de la revendication 1 ou de la revendication 2, dans laquelle le monomère N-(hydroxyalkyl)- ou N-(alcoxyalkyl)-acrylamide renferme un groupe alkyle ayant un nombre d'atomes de carbone inférieur ou égal à 4.

4. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-3, dans laquelle le revêtement réticulé renferme une substance riche en hydroxyles comprenant deux ou plus de deux groupes hydroxyles par molécule ou par unité, dans laquelle la quantité de la substance riche en hydroxyles dans le revêtement réticulé est telle que le ratio du polymère sur la substance riche en hydroxyles est compris entre 100:1 et 1:100.

5. Membrane microporeuse chargée négativement de la revendication 4, dans laquelle la substance riche en hydroxyles est un polysaccharide.

6. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-5, dans laquelle le groupe chargé négativement est un groupe acide sulfonique ou carboxylique.

7. Membrane microporeuse chargée négativement de la revendication 2, dans laquelle le monomère acrylique est un acrylate ou un acrylamide.

8. Membrane microporeuse chargée négativement de
la revendication 7, dans laquelle le monomère acrylique est un acrylamide.

9. Membrane microporeuse chargée négativement de la revendication 8, dans laquelle l'acrylamide est un alkylacrylamide.

10. Membrane microporeuse chargée négativement de la revendication 9, dans laquelle l'acrylamide comprend un groupe acide sulfonique.

11. Membrane microporeuse chargée négativement de la revendication 10, dans laquelle l'acrylamide est un acide acrylamido-N-alkylsulfonique.

12. Membrane microporeuse chargée négativement de la revendication 9, dans laquelle l'alkylacrylamide comprend un groupe acide carboxylique.

13. Membrane microporeuse chargée négativement de la revendication 12, dans laquelle le mélange renferme un monomère acrylique supplémentaire comprenant un groupe acide carboxylique.

14. Membrane microporeuse chargée négativement de la revendication 13, dans laquelle le monomère acrylique supplémentaire est un acrylate.

15. Membrane microporeuse chargée négativement de la revendication 14, dans laquelle l'acrylate est l'acrylate de β-carboxyéthyle.

16. Membrane microporeuse chargée négativement de la revendication 4, dans laquelle le monomère acrylique est un monomère hydroxyacrylique.

17. Membrane microporeuse chargée négativement de la revendication 16, dans laquelle le monomère hydroxyacrylique est un hydroxyacrylamide ou un hydroxyacrylate.

18. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-17, dans laquelle le mélange renferme un N-(alcoxyalkyl) acrylamide.

19. Membrane microporeuse chargée négativement de la revendication 18, dans laquelle le N-(alcoxyalkyl)acrylamide est le N-isobutoxyméthylacrylamide.

20. Membrane microporeuse chargée négativement de la revendication 5, dans laquelle le polysaccharide est le dextran.

21. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-20, dans laquelle la membrane a une capacité à se lier avec des protéines en dynamique supérieure ou égale à environ 25 mg/ml de lysozyme.

22. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-21, dans laquelle le substrat comprend un polymère choisi dans le groupe constitué de polyaramatiques, de polysulfones, de polyléfines, de polystyrènes, de polyamides, de polyimidés, d'acétates de cellulose, de nitrates de cellulose, de polycarbonates, de polyesters et de polymères fluorés.

23. Membrane microporeuse chargée négativement de la revendication 22, dans laquelle le substrat comprend un polysulfone.

24. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-23, dans laquelle le substrat poreux est hydrophile.

25. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-24, dans laquelle une unité insaturée comprenant un groupe chargé négativement forme 0,1 % à 20 % en poids/poids du revêtement réticulé.

26. Membrane microporeuse chargée négativement de l'une quelconque des revendications 1-25, dans laquelle le substrat poreux est hydrophile.

27. Procédé pour préparer une membrane microporeuse chargée négativement de la revendication 1 comprenant un substrat poreux et un revêtement réticulé renfermant des groupes chargés négativement, comprenant :

(a) de fournir un substrat poreux ;
(b) de former un mélange comprenant un monomère insaturé qui comprend un groupe chargé négativement, un monomère insaturé non ionique hydrophile et un monomère N-(hydroxyalkyl)- ou N-(alcoxyalkyl)-acrylamide et de polymériser le mélange pour former un polymère ;
(c) de mettre en contact le substrat avec le polymère ; et
(d) de réticuler le substrat obtenu en (c) pour obtenir la membrane microporeuse chargée négativement comprenant un revêtement réticulé renfermant des groupes chargés négativement.

28. Procédé de la revendication 27, dans lequel le groupe chargé négativement est un groupe acide sulfo-
nique ou carboxylique.

29. Procédé de la revendication 27 ou de la revendication 28, dans lequel le monomère insaturé qui comprend un groupe chargé négativement est un monomère acrylique comprenant un groupe acide sulfonique ou carboxylique.

30. Procédé de la revendication 29, dans lequel le monomère acrylique comprenant un groupe acide sulfonique ou carboxylique est un acrylate ou un acrylamide.

31. Procédé de la revendication 27, dans lequel le procédé comprend de mettre en contact le substrat avec une substance riche en hydroxyles comprenant deux ou plus de deux groupes hydroxyles par molécule avant que le substrat ne soit réticulé de sorte que la quantité de la substance riche en hydroxyles dans le revêtement réticulé soit telle que le ratio du polymère sur la substance riche en hydroxyles dans la membrane est compris entre 100:1 et 1:100.

32. Procédé pour transférer une substance biologique provenant d’un gel d’électrophorèse comprenant de mettre en contact le gel d’électrophorèse avec la membrane de l’une quelconque des revendications 1-26 et de transférer du gel vers la membrane une protéine, un polypeptide, un acide aminé, un acide nucléique ou une association de n’importe lesquels de ceux-ci.

33. Procédé pour séparer d’un fluide des biomolécules chargées positivement, les biomolécules étant choisies dans le groupe constitué de protéines, d’acides aminés, d’acides nucléiques et de virus, le dit procédé comprenant de placer ledit fluide en contact avec la membrane microporeuse chargée négativement de l’une quelconque des revendications 1 à 26 de façon à adsorber sur ladite membrane les biomolécules chargées positivement.
Figure 1
Figure 2

![Graph showing protein (μg/ml) over time (min) with a steep increase starting at 15 minutes.](image-url)
Figure 3

![Graph showing protein concentration over time.](image)