



(11)

EP 3 302 784 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:

06.10.2021 Bulletin 2021/40

(21) Application number: **16804510.2**

(22) Date of filing: **03.06.2016**

(51) Int Cl.:

B01J 20/00 ^(2006.01)	G01N 30/00 ^(2006.01)
B01D 15/08 ^(2006.01)	B01J 20/32 ^(2006.01)
B01J 20/28 ^(2006.01)	B01J 20/10 ^(2006.01)
B01D 15/12 ^(2006.01)	B01J 20/16 ^(2006.01)
B01J 20/30 ^(2006.01)	B01J 20/26 ^(2006.01)

(86) International application number:

PCT/US2016/035683

(87) International publication number:

WO 2016/196906 (08.12.2016 Gazette 2016/49)

(54) **ADSORBENT BIOPROCESSING CLARIFICATION AGENTS AND METHODS OF MAKING AND USING THE SAME**

ADSORBIERENDE KLÄRMITTEL FÜR BIOPROZESSTECHNIK UND VERFAHREN ZUR
HERSTELLUNG UND VERWENDUNG DAVON

AGENTS DE CLARIFICATION ADSORBANTS POUR LE BIOTRAITEMENT ET PROCÉDÉS DE
PRODUCTION ET D'UTILISATION DESDITS AGENTS

(84) Designated Contracting States:

**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR**

(30) Priority: **05.06.2015 US 201562171669 P**

(43) Date of publication of application:

11.04.2018 Bulletin 2018/15

(73) Proprietor: **W.R. Grace & Co.-Conn.**

Columbia, MD 21044 (US)

(72) Inventors:

- **CHODAVARAPU, Surya Kiran**
Ellicott City, Maryland 21042 (US)
- **GU, Feng**
Ellicott City, Maryland 21042 (US)

(74) Representative: **BRP Renaud & Partner mbB**

Rechtsanwälte Patentanwälte
Steuerberater
Königstraße 28
70173 Stuttgart (DE)

(56) References cited:

WO-A1-2011/012302	US-A- 4 576 927
US-A- 4 661 248	US-A- 5 912 037
US-A- 5 993 935	US-A1- 2011 017 670
US-A1- 2014 367 338	US-A1- 2014 367 338

- **GU ET AL.: 'Silica-based strong anion exchange media for protein purification' J. CHROMATOGR. A vol. 1376, 08 December 2014, pages 53 - 63, XP055334221**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

FIELD OF THE INVENTION

[0001] The present invention is directed to adsorbent clarification agents suitable for use in bioprocessing procedures. The present invention is further directed to methods of making and using adsorbent clarification agents for the removal of or separation of undesirable biological substances during bioprocessing procedures.

BACKGROUND

[0002] In recent years biopharmaceutical manufacturing has demonstrated major improvements in MAb production, exhibiting product titers as high as 25 g/L often associated with very high cell densities. High-density cell cultures with >150 million cells/mL pose a great challenge in further downstream processing because of a need to remove a large amount of biomass and increased levels of contaminants from cell debris generated during cell culture and harvesting. Production of biological substances (MAbs, in particular) usually involves processing a complex cell culture broth from which desired biological substances must be isolated and purified while maintaining high overall product recovery and quality. Traditionally, centrifugation and a combination of filtration techniques (tangential-flow filtration and depth filtration) have been widely accepted as workhorses for clarifying these complex cell culture broths. However, improvement of mammalian cell culture processes is providing for total cell densities far beyond traditional levels of 20×10^6 cells/mL for CHO cells to $>150 \times 10^6$ cells/mL for PER.C6 cells. Thus, limitations of both centrifugation and filtration techniques are apparent by the high ($\leq 40\%$) solids content of such harvests.

[0003] Centrifugation can be applied to process feed streams with high levels of solids, for instance. However, product recovery can be low because of increased pellet volumes and a need to desludge frequently (especially in large-scale continuous centrifugation). Additionally, cell disruption from shear forces generated during centrifugation can further decrease the efficiency of harvest clarification and potentially cause product damage and/or entrapment.

[0004] Depth filters are advantageous because they remove contaminants, and many come in single-use format, reducing the need for cleaning and validation. However, depth filters are currently unable to handle high-solids feed-streams and are often used in series with centrifugation. TFF can handle high solids loading, but this technique can exhibit poor yield because of polarization of solids at the membrane surface when processing highly dense feed streams. Excessive product dilution and cell lysis caused by shear forces can also limit the utility of TFF.

[0005] Flocculation of cell culture harvests has also been widely used to enhance clarification throughput and downstream filtration operations. Current techniques include the use of soluble polyionic polymers (such as DEAE dextran, acryl-based polymers, and polyethylene amine) and inorganic materials such as diatomaceous earth and perlites, which remove cells and cell debris. However, polymers must subsequently be removed from process streams, which requires monitoring and quantification by in-process and product-release assays. If IEX chromatography is included as a purification step in the downstream process, binding capacities will be greatly affected by the charged nature of flocculants. The high viscosity of polycation stock solutions presents an additional process challenge.

[0006] In recent years, various new clarification techniques have been developed. For example, (<http://www.select-science.net/product-news/sartorius-stedim-biotech>) single use harvesting technology for high cell density cultures using diatomaceous earth (DE) as filter aid has been introduced. (Minow B., et al, BioProcess Int. , April 1st, 2014). However, given its low surface area (BET ~ 1 m²/g) and non-porous nature, DE has no function in adsorption of proteins and/or other types of biologics, but only as filter aid.

[0007] In other processes, functionalized particulate materials have been used as adsorbents for clarification of cell broths. For example, WO2010043700 and WO2010043703 methods of clarification or removing host cell proteins by the utilization of anion exchange material in the process. The property or optimal conditions for the ion exchange materials are not described in the patent applications. US2014/367338, US4576927 and WO2011/012302 mention the use of silica particles with polymeric material covalently bonded to their surfaces as bioprocessing clarification agents.

[0008] Efforts continue to further develop clarification materials and processes having improved agents in the form of functionalized particulate materials so as to improve binding capacity (i.e., the amount and the variety of biomaterials that can be bound to the adsorbent clarification agents) and/or binding selectivity of the functionalized particulate materials.

SUMMARY

[0009] The present invention addresses the need in the art for improved bioprocesses by the discovery that the use of certain functionalized porous silica materials provide enhanced performance as clarification adsorbents. The adsorbents of the present invention unexpectedly provide exceptional binding capacity, as well as binding selectivity in bioprocesses (e.g., biopurification, bioseparation, etc.) when compared to known functionalized particulate materials.

[0010] In particular, the present invention provides improved bioprocesses employing polymeric functionalized silica materials comprised of porous silica particles having a sufficiently wide pore size distribution to enable the particles to capable of selectively bind to a wide variety of biomolecules including, but not limited to, cells, host cell proteins, nucleic acids, cell debris, chromatins, etc., so as to remove these materials during one or more bioprocessing steps. The porous silica particles have a pore size distribution as measured by a pore size distribution span 90 value of at least 2.8. In other embodiments, wherein the porous particles having a pore size distribution as measured by a pore size distribution span 90 value of as much as about 5.0 to about 10.0.

[0011] Accordingly, the present invention is directed to polymer functionalized porous silica particles having a specified pore size distribution (also referred to herein as "adsorbents") which are useful as clarification agents in a variety of bioprocesses. The present invention is directed to adsorbents comprising polymeric material covalently bonded to the surface of porous silica particles wherein said particles have a pore size distribution as measured by a pore size distribution span 90 value of at least 2.8. In some desired embodiments, the invention comprise the adsorbents useful as a bioprocessing clarification agent, wherein the adsorbents comprise a polyelectrolyte material covalently bonded to the surface of porous silica particles, wherein the porous particles have a pore size distribution as measured by a pore size distribution span 90 value of as much as about 5.0 to about 10.0. In preferred embodiments of the invention, the polymeric material comprises a polyelectrolyte, e.g. polyethyleneimine.

[0012] The present invention is even further directed to methods of making adsorbents in the form of functionalized particulate material. In one exemplary embodiment, the method of making adsorbents of the present invention comprises: contacting porous silica particles with one or more reactants under reaction conditions that result in at least one polymeric material covalently bonded to at least one surface of the silica particles, wherein the silica particles have a wide pore size distribution as measured by a pore size distribution span 90 value of at least 2.8.

[0013] The present invention is also directed to a method for the clarification of a bioprocess mixture during a bioprocessing procedure. The method provides for the removal or separation of the undesirable biological substances, e.g. cell debris, from a bioprocessing mixture by using the adsorbents of the invention.

[0014] These and other features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIGURE graphically shows the comparison in pore size distributions (from mercury porosimetry) for two different silicas.

DETAILED DESCRIPTION

[0016] To promote an understanding of the principles of the present invention, descriptions of specific embodiments of the invention follow and specific language is used to describe the specific embodiments. It will nevertheless be understood that no limitation of the scope of the invention is intended by the use of specific language. Alterations, further modifications, and such further applications of the principles of the present invention discussed are contemplated as would normally occur to one ordinarily skilled in the art to which the invention pertains.

[0017] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an oxide" includes a plurality of such oxides and reference to "oxide" includes reference to one or more oxides and equivalents thereof known to those skilled in the art, and so forth.

[0018] "About" modifying, for example, the quantity of an ingredient in a composition, concentrations, volumes, process temperatures, process times, recoveries or yields, flow rates, and like values, and ranges thereof, employed in describing the embodiments of the disclosure, refers to variation in the numerical quantity that may occur, for example, through typical measuring and handling procedures; through inadvertent error in these procedures; through differences in the ingredients used to carry out the methods; and like proximate considerations. The term "about" also encompasses amounts that differ due to aging of a formulation with a particular initial concentration or mixture, and amounts that differ due to mixing or processing a formulation with a particular initial concentration or mixture. Whether modified by the term "about" the claims appended hereto include equivalents to these quantities.

[0019] As used herein, the terms "bioprocess" or "bioprocessing" refer to a batch process that forms, alters and/or utilizes one or more biomolecules. The term "bioprocess" includes, but is not limited to, bioseparation processes, biopurification processes, processes for forming biopharmaceuticals, and processes for purifying biopharmaceuticals. As used herein, the term "bioprocess" or "bioprocessing" refers to processing that may take place prior to a chromatography process (e.g., removing cell debris from a batch of material), does not encompass chromatography processes (e.g., liquid chromatography).

[0020] As used herein, the term "biomolecule" means any molecule that is produced by a living organism, including

large molecules such as proteins, polysaccharides, lipids, and nucleic acids; and small molecules such as primary metabolites, secondary metabolites, and natural products. Examples of biomolecules include cells and cell debris; antibodies, proteins and peptides; nucleic acids, such as DNA and RNA; endotoxins; viruses; vaccines and the like. Other examples of biomolecules include those recited in WO 2002/074791 and U.S. 5,451,660.

[0021] As used herein, the term "functionalized" as it relates to the surface of the silica particles means porous silica particles that have been surface modified by reaction with at least one functional compound to alter the selectivity of at least a portion of the particle surface, including the surface area on the external portion of the particles, and/or on the surface area of the internal pores. The functionalized surface of the particles may be used to form a bonded phase (covalently or ionically), a coated surface (e.g., reverse phase C18 bonded), a polymerized surface (e.g., ion exchange), an inherent surface (e.g., inorganic/organic hybrid material), or the like. For example, reacting porous inorganic oxide particles with octadecyltrichlorosilane forms a "reverse phase" by covalently bonding the silane to the inorganic surface (e.g., C4, C8, C18, etc.). In another example, reaction of the inorganic particles with aminopropyltrimethoxysilane followed by quaternization of the amino group forms an "anion exchange phase." In a third example, a bonded phase may be formed by reaction of the inorganic particles with aminopropyltrimethoxysilane followed by formation of an amide with an acid chloride. Other bonded phases include diol, cyano, cation, affinity, chiral, amino, C18, hydrophilic interaction (HILIC), hydrophobic interaction (HIC), mixed mode, size exclusion, etc. As part of the bonded phase or functionalized surface, a ligand may be used to show specific interaction with the target molecule or biomolecule (e.g., ligate), such as those set forth in U.S. 4,895,806.

[0022] As used herein, the term "polyelectrolyte" is defined as meaning a polymer comprising repeating units such as (i) a cation, (ii) an anion, or (iii) an electrolyte functional group that associates or dissociates with a proton in aqueous solutions to form a positive or negative charge. For example, cationic polyelectrolytes suitable for use in the present invention include, but are not limited to, polyethyleneimine (PEI), polyallylamine, polyvinyl pyridine, polydiallyldimethylammonium chloride (pDADMAC), and copolymers containing similar functional groups. Anionic polyelectrolytes suitable for use in the present invention include, but are not limited to, polyacrylic acids, polymethacrylic acids, polystyrene sulfonic acids, nucleic acids, and copolymers containing similar functional groups (e.g., poly(styrene-co-maleic acid, poly(styrene sulfonic acid-co-maleic acid)).

[0023] As used herein, the term "polyethyleneimine" is defined as meaning a polymer comprising repeating units of an amine bonded to a $-CH_2CH_2-$ spacer, wherein the polymer may be linear or branched and may contain primary, secondary and/or tertiary amino groups.

[0024] As used herein, the term "BET particle surface area" is defined as meaning a particle surface area as measured by the Brunauer Emmet Teller nitrogen adsorption method.

[0025] As used herein, the term median pore diameter (size) distributions were measured by mercury intrusion using an Autopore® IV 9520, available from Micromeritics Instrument Corporation (Norcross, GA).

[0026] As used herein, the term "pore size distribution" means the relative abundance of each pore size in a representative volume of porous inorganic particles.

[0027] As used herein "median pore diameter" is the Median pore diameter (PD50) is defined as the midpoint at which 50% of the pore volume is contributed by smaller pores and 50% is contributed by larger pores.

[0028] As used herein, the term "pore volumes" referenced herein represent cumulative volumes from mercury intrusion into 50-10,000 Å size pores.

[0029] As used herein, the term "molecular weight" is defined as meaning the molar mass of a single molecule of a particular compound or polymer.

[0030] As used herein, the "pore size distribution span 90 value" is calculated as $[(PD90 - PD10)/PD50]$, whereas PD10 is the pore diameter at 10% cumulative pore volume contribution, PD50 is the pore diameter at 50% cumulative pore volume contribution, and PD90 is the pore diameter at 90% cumulative pore volume contribution, all based on mercury intrusion data.

Porous Particles

[0031] As discussed above, the porous silica particles used to prepare the adsorbents of the present invention possess a wide pore size distribution as measured by a pore size distribution span 90 value of at least 2.8, or at least about 3.0, or at least about 4.0, or at least about 5.0, or at least about 6.0, or at least about 7.5, desirably, from about 5.0 to about 10.0, more desirably, from about 7.5 to about 8.5, and in some embodiments, about 7.8). The wide pore size distribution enables the formation of adsorbent clarification agents that provide exceptional binding capacity, binding selectivity, as well as binding of a wide variety of biomolecules.

[0032] Generally, the porous silica particles have a pore size distribution of from about 30 to about 5000 Å.

[0033] Advantageously, the wide pore size distribution of the porous silica particles used to form the adsorbents of the present invention enables controlled binding capacity, binding selectivity, and binding of a wide variety of biomolecules by choosing (1) specific polymeric material bound to surfaces of the silica particles, (2) the amount of polymeric material

bound to surfaces of the silica particles, and/or (3) the molecular weight of the polymeric material bound to surfaces of the silica particles. As shown and discussed in the example section below, the wide pore size distribution of the silica particles used to form the adsorbents of the present invention provides more flexibility and binding capacity when compared to known, commercially available adsorbents.

[0034] Typically, the porous silica particles useful to prepare the adsorbents of the invention possess a median pore size of at least about 100 Å. In another embodiment, the porous silica particles have a median pore size of at least about 150 Å (or at least about 200 Å; or at least about 250, or at least about 300 Å; or at least about 350 Å; or at least about 400 Å, or at least about 500 Å; or at least about 600 Å; or at least about 800 Å. In some embodiments, the porous silica particles have a median pore size of from about 100 Å to about 800 Å. In some embodiments, the porous silica particles have a median pore size of from about 150 Å to about 250 Å. In other embodiments, the porous silica particles have a median pore size of less than 2000 Å. In other embodiments, the porous silica particles have a median pore size of less than 1000 Å.

[0035] The silica particles typically have a particle size, as measured by a median particle dimension, of less than 150 µm. The porous silica particles typically have a median particle dimension of about 1 µm, more typically, less than about 100 µm. In some embodiments, the porous silica particles have a median particle dimension of from about 10 to about 50 µm, more desirably, about 30 µm.

[0036] Typically, the silica particles typically have an irregular shape, but may have any shape (e.g., spherical, elliptical, etc.). Regardless of shape, the porous silica particles typically have a median particle dimension as discussed herein-above.

[0037] In additional embodiments, the porous silica particles may have an aspect ratio of at least about 1.0 as measured, for example, using Transmission Electron Microscopy (TEM) techniques. As used herein, the term "aspect ratio" is used to describe the ratio between (i) the median particle dimension of the porous silica particles and (ii) the median cross-sectional particle dimension of the porous silica particles, wherein the cross-sectional particle dimension is substantially perpendicular to the largest particle dimension of the porous silica particles. In some embodiments of the present invention, the porous silica particles have an aspect ratio of at least about 1.1 (or at least about 1.2, or at least about 1.3, or at least about 1.4). Typically, the porous silica particles have an aspect ratio of from about 1.0 to about 1.5.

[0038] The pore volume of the porous silica particles as measured by mercury intrusion into 50-10,000 Å size pores. In another embodiment, the silica particles as measured by mercury intrusion into 50-10,000 Å size pores - is at least about 0.25 cc/g 0.50 cc/g. In one exemplary embodiment of the present invention, the porous silica particles have a pore volume as measured by mercury intrusion into 50-10,000 Å size pores - of from at least about 1.0 cc/g ; at least about 1.50 or at least about 1.77 cc/g. In another exemplary embodiment of the present invention, the porous silica particles have a pore volume as measured by mercury intrusion into 50-10,000 Å size pores - of from about 1.0 cc/g to about 3.0 cc/g.

[0039] The surface area of porous silica particles also have a surface area as measured by the BET nitrogen adsorption method (i.e., the Brunauer Emmet Teller method) of at least about 100 m²/g, or at least about 300 m²/g, or at least about 500 m²/g. In one exemplary embodiment of the present invention, the porous silica particles have a BET surface area of from about 100 m²/g to about 1000 m²/g, or from 600 m²/g to about 800 m²/g. In a further exemplary embodiment of the present invention, the porous silica particles have a BET surface area of from about 700 m²/g.

[0040] The particles desirably comprise silica having a purity of at least about 93% by weight SiO₂, or at least about 93% by weight SiO₂, at least about 94% by weight SiO₂, at least about 95% by weight SiO₂, at least about 96% by weight SiO₂, at least about 97% by weight SiO₂, or at least about 98% by weight SiO₂ up to 100% by weight SiO₂ based upon the total weight of the particle.

Adsorbents

[0041] In accordance with the present invention, the adsorbent comprises porous silica particles as described herein above and a polymeric material covalently bonded to surfaces of said silica particles. In one embodiment the adsorbents comprises at least one bifunctional moiety extending from said surfaces of said silica particles, each of said at least one bifunctional moiety comprising (i) one or more functional groups capable of bonding to said surfaces, and either (iia) said polymeric material, or (iib) one or more reactive groups capable of bonding to said polymeric material.

[0042] In another embodiment, the adsorbents comprises at least one bifunctional moiety extending from the surfaces of said silica particles, each of said at least one bifunctional moiety comprising (i) one or more functional groups capable of bonding to said surfaces, and (iia) said polymeric material.

[0043] In yet another embodiment, the adsorbents comprises at least one bifunctional moiety extending from said surfaces of said silica particles, each of said at least one bifunctional moiety comprising (i) one or more functional groups capable of bonding to said surfaces, and (iib) one or more reactive groups capable of bonding to said polymeric material.

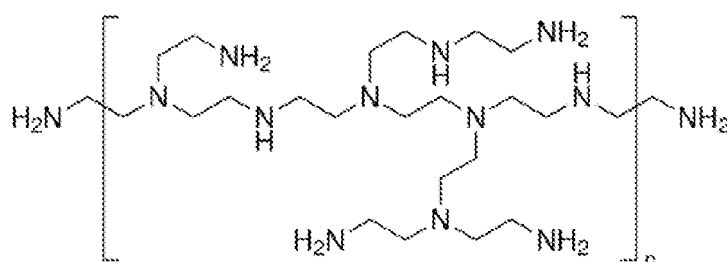
[0044] Suitable bifunctional moiety extending from the surface of said silica particle comprises epoxy rings that can react with amine groups from the polymer chains of the polyelectrolytes. In one embodiment, the at least one bifunctional

moiety comprising the adsorbent comprises product from reaction of silica surface with an epoxy silane. In a preferred embodiment, the epoxy silane comprises (3-glycidioxypropyl)-trimethoxysilane.

[0045] In one embodiment of the invention, the polymeric material is covalently bonded to the surface of the silica particles via a covalent bond linkage consisting of one or more atoms selected from the group consisting of C, O, Si and N.

[0046] Typically, the polymeric material comprises a polyelectrolyte. The polyelectrolyte may be cationic or anionic. Suitable cationic polyelectrolyte materials comprise polyethyleneimine, polyallylamine, polyvinyl pyridine, polydiallyldimethylammonium chloride (pDADMAC), a copolymer containing one or more cationic or cationic-forming functional groups, or combinations thereof. The cationic polyelectrolyte may also comprise a reaction product of said metal oxide particles and an aqueous trimethoxysilyl-polyethyleneimine solution. In a preferred embodiment, the polymeric material comprises polyethyleneimine. In even more preferred embodiment, the polyethyleneimine have (i) a weight average molecular weight, Mw, and (ii) a number average molecular weight, Mn, each of which is less than about 5000. In still a more preferred embodiment, the polyethyleneimine has (i) a weight average molecular weight, Mw, and (ii) a number average molecular weight, Mn, each of which ranges from about 500 to about 2500; or. (i) a weight average molecular weight, Mw, ranging from about 800 to about 2000, and (ii) a number average molecular weight, Mn, ranging from about 600 to about 1800.

[0047] In one embodiment, the polymeric material comprises a polyethyleneimine has a structure:



prior to being covalently bonded to said surfaces, and n is a number greater than or equal to 1.

[0048] Suitable anionic polyelectrolyte materials comprise polyacrylic acid, polymethacrylic acid, polystyrene sulfonic acid, nucleic acid, a copolymer containing one or more anionic or anionic-forming functional groups or combinations thereof. In a preferred embodiment, the anionic polyelectrolyte material comprises polyacrylic acid, polymethacrylic acid, or a combination thereof. In a more preferred embodiment, the anionic polyelectrolyte material comprises a polyacrylic acid or a polymethacrylic acid having a weight average molecular weight, Mw, greater than about 50,000. In an even more preferred embodiment, the polyacrylic acid or polymethacrylic acid has a weight average molecular weight, Mw, of from about 100,000 to about 250,000.

[0049] The amount of the polymeric material present in the adsorbent is any amount suitable to provide charge interactions with biologics and therefore selectively bind to these biologics of opposite charge. In one embodiment, the polymeric materials comprise an amount such that less than 100% of said surfaces of the silica particles are bonded to said polymeric material. In another embodiment, said the polymeric material is present on the adsorbent in an amount representing up to about 20.0 wt% of said adsorbents based on a total weight of said adsorbents. In other embodiments, the polymeric material is present in an amount representing from about 1.0 to about 15.0 wt% of said adsorbents based on a total weight of said adsorbents.

Methods of Making Adsorbents

[0050] The adsorbent of the invention are generally prepared by contacting the silica particles with one or more reactants under reaction conditions that result in covalently bonding the polymeric material to the surfaces of the silica particles. The contacting step may utilize a solvent. In one embodiment, the contacting step utilizes a reaction mixture free of organic solvent.

[0051] In one embodiment of the invention, the one or more reactants comprise at least one bifunctional compound comprising (i) one or more functional groups capable of bonding the compound to the surfaces of the silica particles, and either (iia) the polymeric material, or (iib) one or more reactive groups capable of bonding to the polymeric material.

[0052] Suitable reactants comprising at least one bifunctional compound comprising (i) one or more functional groups capable of bonding the compound to the surfaces of the silica particles, and (iia) the polymeric material include, but is not limited to, polyethyleneimines.

[0053] Suitable reactants comprising at least one bifunctional compound comprising (i) one or more functional groups capable of bonding the compound to the surfaces of the silica particles, and (iib) one or more reactive groups capable of bonding to the polymeric material, include but is not limited to, polyacrylic acids.

[0054] It is also within the scope of the invention to choose the one or more reactants so as to produce adsorbents having a desired degree of affinity for one or more particular biological compounds through attraction due to opposite charges. For example, the affinity of negatively charged DNA towards positively charged surface. The amount of each of the one or more reactants may also be chosen so as to form adsorbents having a desired degree of particle surface coverage by the polymeric material.

Use of Adsorbents

[0055] Adsorbent in accordance with the present invention comprise silica surface functionalized with polyelectrolytes. To achieve the adsorption of higher amount of undesirable biological substances, the requirements of the porous molecules are high pore volume (>1.0 cc/g) and high pore size distribution span 90 value (>1.0). If these conditions are met, with the bonding of polyelectrolyte, either positively or negatively charged, the adsorbent materials can be effectively used for use as both adsorbents and clarification agents. In one embodiment, the functionalized adsorbents are used as a bioprocessing clarification agent, wherein the adsorbents comprises: silica particles having a wide pore size distribution as measured by a pore size distribution span 90 value of at least 2.8; and polymeric material covalently bonded to surfaces of the metal oxide particles.

[0056] The adsorbent may be used by contacting the functionalized porous adsorbent in a bioprocessing vessel containing undesirable biologics obtained during a bioprocessing procedure; allowing a sufficient incubation time to permit adsorption of the undesirable biologics to the adsorbent or formation of cell pellet and a supernatant layer; separating the resulting mixture through either filtration or decanting or pumping out the supernatant layer and thereafter extracting the desired biological substance from either the filtrates or supernatant or carrying out further purification steps including depth filtration and downstream chromatography steps.

[0057] In one embodiment, the adsorbents in accordance with the present invention are added to a bioprocessing vessel containing one or more biomolecules selected from the group consisting of a protein, a polysaccharide, a lipid, a nucleic acid, a metabolite, a mammalian cell, mammalian cell debris, an antibody, a peptide, DNA, RNA, an endotoxin, a virus, a vaccine, an enzyme, or any combination thereof. In other embodiment, the bioprocessing vessel contain at least one of: host cells, cell debris, host cell proteins, nucleic acids, chromatins, antibodies, virus, or vaccines. The functionalized adsorbents bind to one or more biomolecules that come into contact with the adsorbents. Following the binding step in which the adsorbents binds to one or more biomolecules that come into contact with the adsorbents, the adsorbents bound to the one or more biomolecules is separated from other components of the bioprocessing procedure.

[0058] It should be understood that although the above-described adsorbents and methods are described as "comprising" one or more components or steps, the above-described adsorbents and methods may "comprise," "consists of," or "consist essentially of" any of the above-described components or steps of the adsorbents and methods. Consequently, where the present invention, or a portion thereof, has been described with an open-ended term such as "comprising," it should be readily understood that (unless otherwise stated) the description of the present invention, or the portion thereof, should also be interpreted to describe the present invention, or a portion thereof, using the terms "consisting essentially of" or "consisting of" or variations thereof as discussed below.

[0059] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains," "containing," "characterized by" or any other variation thereof, are intended to encompass a non-exclusive inclusion, subject to any limitation explicitly indicated otherwise, of the recited components. For example, an adsorbent and/or method that "comprises" a list of elements (e.g., components or steps) is not necessarily limited to only those elements (or components or steps), but may include other elements (or components or steps) not expressly listed or inherent to the adsorbent and/or method.

[0060] As used herein, the transitional phrases "consists of" and "consisting of" exclude any element, step, or component not specified. For example, "consists of" or "consisting of" used in a claim would limit the claim to the components, materials or steps specifically recited in the claim except for impurities ordinarily associated therewith (i.e., impurities within a given component). When the phrase "consists of" or "consisting of" appears in a clause of the body of a claim, rather than immediately following the preamble, the phrase "consists of" or "consisting of" limits only the elements (or components or steps) set forth in that clause; other elements (or components) are not excluded from the claim as a whole.

[0061] As used herein, the transitional phrases "consists essentially of" and "consisting essentially of" are used to define a adsorbents and/or method that includes materials, steps, features, components, or elements, in addition to those literally disclosed, provided that these additional materials, steps, features, components, or elements do not materially affect the basic and novel characteristic(s) of the claimed invention. The term "consisting essentially of" occupies a middle ground between "comprising" and "consisting of".

[0062] Further, it should be understood that the herein-described adsorbents and/or methods may comprise, consist essentially of, or consist of any of the herein-described components and features, as shown in the figures with or without any feature(s) not shown in the figures. In other words, in some embodiments, the adsorbents and/or methods of the present invention do not have any additional features other than those shown in the figures, and such additional features,

not shown in the figures, are specifically excluded from the adsorbents and/or methods. In other embodiments, the adsorbents and/or methods of the present invention do have one or more additional features that are not shown in the figures.

[0063] The present invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the scope of the appended claims.

EXAMPLES

[0064] Median particle sizes were determined by laser light scattering using a Malvern Mastersizer 2000, available from Malvern Instrument Ltd. (per ASTM B822-10). Particle size is defined as median particle size by volume distribution. BET surface areas were obtained from the nitrogen sorption analysis described in the literature. Median pore size, pore volume and pore size distribution were calculated based on mercury intrusion into 35-10000 Å size pores. Pore volume is defined as cumulative pore volume in the same pore size range, and median pore size is determined a pore diameter (size) at which 50% pore volume is contributed from smaller pores and 50% pore volume is contributed from bigger pores. Span 90 is defined as a relative pore size span and calculated as (PD90 - PD10)/PD50, whereas PD10 is pore diameter at 10% cumulative pore volume contribution and PD50 and PD90 is as defined as 50% and 90% cumulative pore volume contributions based on mercury data, respectively.

1. Polyethyleneimine (PEI) bonding for cationic surface

[0065] Six different grades of silica (A, B, C, D, E, F), available from W. R. Grace, were used in the these examples, and their properties (BET Surface Area, Pore Volume, Pore size distribution Span 90, and median pore size PD50) are listed in the Table I below:

Table I

Silica	Surface Area (m ² /g)	Pore Volume (cc/g)	Span 90	PD50 (Å)
A	699	1.77	7.80	432
B	253	1.19	2.80	154
C	79	1.21	1.25	500
D	711	0.10	0.94	90
E	314	0.57	1.10	569
F	713	0.58	0.93	104
G	247	0.18	0.43	50
H	1	0	0	0

[0066] The particle size of these silica ranges from 5 µm to 70 µm. Silica B is commercial silica with a Trade name of Syloid® W900, Silica C is a commercial silica with a Trade name of Davisil® XWP 500, Silica F is a commercial silica with a Trade name of Trisyl®. All these are available from W. R. Grace & Co. Silica G is a commercially available (Polyethyleneimine on silica gel, powder, 40-200 mesh from Sigma-Aldrich Corporation) PEI bonded silica sample. Silica H in the Table is a diatomaceous earth (powder, suitable for most filtrations, from Sigma-Aldrich Corporation) and the bonding with PEHA was attempted using the same procedure as the other samples.

[0067] Silica A, B had the properties of Pore Volumes of greater than 1.0 cc/g and Span 90 values of greater than 1.0.

[0068] In the examples, pentaethylenehexamine (PEHA) was used, a mixture of small molecule polyamines to represent PEI in our examples.

[0069] The bonding process consisted of two steps: 1). Surface bonding with epoxy silane (3-glycidoxypyrrol-trimethoxysilane), and then 2). Reaction of PEHA with surface epoxy groups (attacking of nucleophilic amino groups to the epoxy ring, and this leads to amine attachment with opening of epoxy ring, forming β-amino alcohols (N. Azizi, M. R. Saidi, Org. Lett., 2005, 7, 3649-3651).

1A. Initial Bonding procedure

[0070] Modified silica samples were prepared by treating the bare silica gel particles (dried in a 120°C oven overnight) with epoxy silane. A 1 L, round bottom indented flask was charged with silica particles (100 g), and then 10 g (10 w/w% to silica) of epoxy silane is added drop-wise into the flask while rotating. The mixture was allowed to roll on a rotovap overnight (16 h). A small portion (2 g) of the sample was separated and washed with 10 ml of acetone five times and dried in a 70°C oven for 20 hours. The dried sample was subjected to elemental analysis for the amount of carbon content by using a LECO instrument.

1B. PEI reaction

[0071] 20g of bonded silica was weighed from the sample obtained in 1A above and placed in an Erlenmeyer Flask. 6 g of PEHA was weighed in a small beaker (30% w/w to silica), and mixed with **100 g** of DIW to dissolve PEHA, to provide ~15% silica slurry for the reaction). The mixture was shaken well and allowed to sit in a water bath (65°C for 30 min). After the reaction, the silica was further diluted with DI water, filter. Then the silica was soaked with 50 ml 0.5 M HCl for 20-30 minutes and filtered. The resulting silica was washed with DIW for three times (3 x 100 ml) and dried a small sample at 90°C overnight. The dried sample was sent for C% analysis. The rest of sample was dried inside the hood in ambient conditions prior to analysis.

1C. Batch Binding Capacity test

[0072] Two model compounds were used in these examples: 1. DNA oligonucleotides (Deoxyribonucleic acid from herring sperm from Sigma-Aldrich), less than 50 base pairs, MW < 15000. 2. Bovine serum albumin (BSA) (lyophilized powder, crystallized, ≥98.0% From Sigma), MW ~ 66000.

[0073] Test method: The binding capacities were performed using BSA solution (5 mg/ml) at a pH of 8.0 with 20 mM Tris-HCl buffer, or DNA solution (2 mg/ml) at pH 3.45 with 10 mM sodium phosphate buffer. Dried silica samples were weighed into vials, and then protein solutions (UV/Vis adsorption was measured at 280 nm using a Genesys®10S Bio UV-Vis spectrophotometer). After 2 hr, the supernatants were collected the adsorptions were again measured under the same conditions. The percentage of UV/Vis signal drop were calculated (the higher drop before and after the adsorption, the greater binding capacity). Three parallel adsorption measurements were carried out for one sample and the averages were calculated and reported.

[0074] The following table shows the binding capacity results of Examples 1-10:

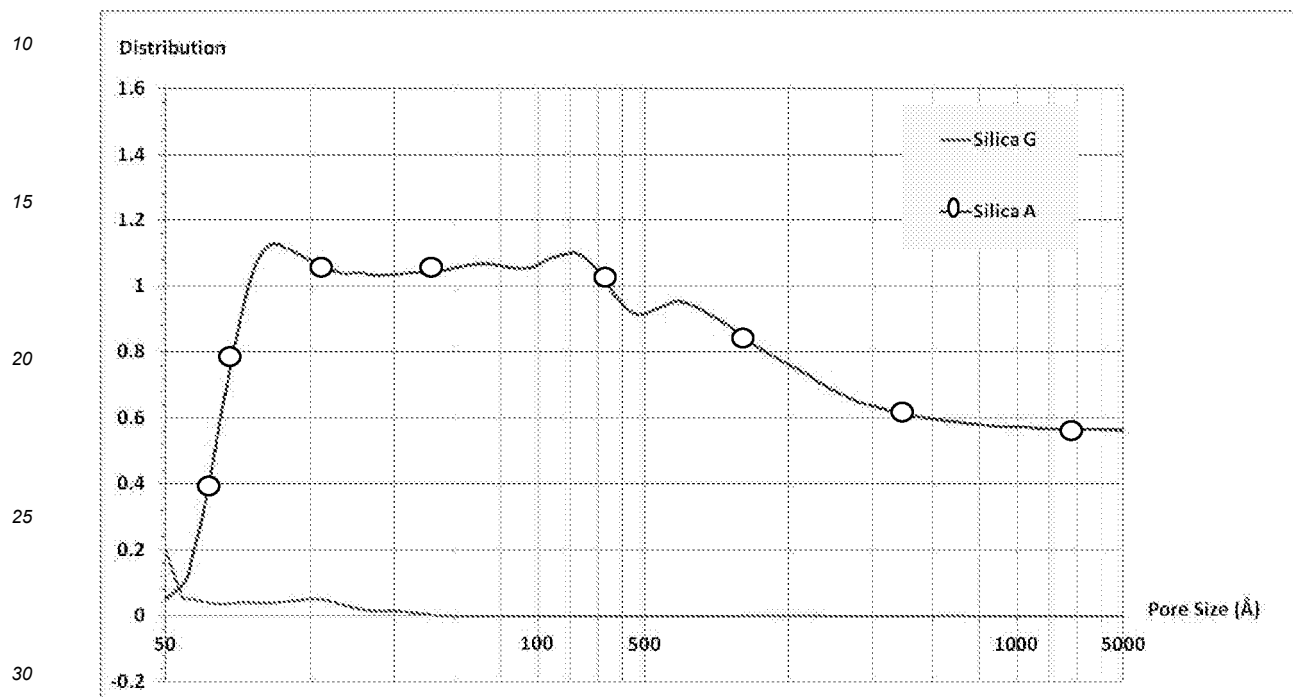
Table 2

Examples	Silica	C%	DNA Binding	BSA Binding
1	A	3.19	94%	33%
2	B	1.70	77%	41%
3	C	1.25	37%	23%
4	D	4.17	89%	0.1%
5	E	0.92	38%	5.6%
7	F	4.06	61%	1.1%
8	G	8.07	8.4%	0.0%
9	H	0.05	2.0%	0.0%
10	A	Unmodified	20%	4.1%

[0075] As mentioned above, Examples 1-6 were made by treating the dried silica with epoxysilane and then followed by reacting the silica intermediates with PEHA. The C% numbers, calculated by subtracting the total C% from the C% from the epoxy bonded silica, are indications of the total amount of amines are attached from the reactions. For Sample 7 (commercial PEI-silica) (BET and Hg measurements were carried out after the surface groups of the sample were burnt off by placing samples in an 460°C muffle oven in air overnight) the binding of DNA and BSA were measured using the same measurement method. The diatomaceous earth (Silica H) for Example 8 was also bonded with PEHA using the same procedure. As shown, Example 8 has very low level of amount of PEHA bonded (due to very low surface area) and the amount of DNA adsorbed.

[0076] The results shown above indicate that in order to get high amount of DNA and BSA adsorbed, high pore volume and span 90 are required. Samples 1, 2, and 3 had both pore volume and Span 90 greater than 1.0, and these samples have showed higher binding of both DNA and BSA. Other samples all showed lower binding. In Example 9, due to its high surface area, unbounded silica A had some binding capability of DNA and BAS, but the binding were very low when compared to PEHA bonded Sample 1.

[0077] The following Figure shows the comparison in pore size distributions (from mercury porosimetry) for Silica A and Silica G. As noted, Silica A has the widest pores size distribution ranging from 50 Å to over 5000 Å.



FIGURE

2. Bonding silica with anionic polymers for binding of basic proteins

2A. Preparation

[0078] The preparation of anionic polymer bonded silica was achieved by mixing the neutral aqueous solution of polymers (neutralized by base such as ammonium hydroxide or sodium hydroxide) with silica, and then baking the mixture at 190°C for 5 hrs to force the bonding formation of the polymer and the surface groups. The solutions of commercial poly(acrylic acid) or poly(methacrylic acid) (from Sigma-Aldrich Corporation) were made by dissolving (or diluting if commercial product is an aqueous solution) polymer in DI water and the pH of the solution is adjusted to 7-8 with ammonium hydroxide (or sodium hydroxide). The concentration for the solutions was about 18%.

[0079] 20 g of silica (Silica A) was mixed with polymer solution containing about 10 g of net polymer. The mixture was placed in a ceramic container and place in an oven in a well ventilated fume hood. The mixture was heated at 90°C until no liquid is seen, and then baked at 190°C for 5 hrs. The resulting mixture was re-slurried with DI water, and washed with 5 x 200 ml of 10% NaCl and 1 x 200 ml of NaHCO₃, followed by 3 x 100 ml DI water.

2B. Protein binding evaluation

[0080] The procedure was similar to procedure as described in 1C, with exception that lysozyme (from chicken egg white, lyophilized powder, protein ≥90 %, ≥40,000 units/mg protein from Sigma) was used in this test, and the concentration of the protein was 25 mg/ml in 50 mM sodium phosphate, pH 7.0. The amount of adsorbed protein was measured through the amount of signal loss after 2 hr incubation at room temperature, from UV/Vis at 280 nm wavelength, and calculated by lysozyme absorption coefficient.

[0081] The following Table 3 shows the protein binding results for Examples 11-14 below:

Table 3.

Example #	Polymer	Polymer MW	C%	Lysozyme Binding (mg/mg)
10	PAA	100000	18.24	1.44
11	PAA	240000	20.55	1.44
12	PAA	15000	5.05	0.67
13	PMA	9500	0.50	0.45
14	None (Control)	None	0.00	0.52

[0082] Table 3 shows that higher lysozyme binding is associated with high molecular weight of polymer. Low molecular weight polymer samples had similar binding to that of control, non-polymer bonded silica (Sample 14).

[0083] While the invention has been described with a limited number of embodiments, these specific embodiments are not intended to limit the scope of the invention as otherwise described and claimed herein. It may be evident to those of ordinary skill in the art upon review of the exemplary embodiments herein that further modifications, equivalents, and variations are possible. All parts and percentages in the examples, as well as in the remainder of the specification, are by weight unless otherwise specified. Further, any range of numbers recited in the specification or claims, such as that representing a particular set of properties, units of measure, conditions, physical states or percentages, is intended to literally incorporate expressly herein by reference or otherwise, any number falling within such range, including any subset of numbers within any range so recited. For example, whenever a numerical range with a lower limit, R_L , and an upper limit R_U , is disclosed, any number R falling within the range is specifically disclosed. In particular, the following numbers R within the range are specifically disclosed: $R = R_L + k(R_U - R_L)$, where k is a variable ranging from 1% to 100% with a 1% increment, e.g., k is 1%, 2%, 3%, 4%, 5%.... 50%, 51%, 52%.... 95%, 96%, 97%, 98%, 99%, or 100%. Moreover, any numerical range represented by any two values of R , as calculated above is also specifically disclosed. Any modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and accompanying drawings, without departing from the scope of the appended claims.

Claims

1. Adsorbents comprising:

silica particles having a wide pore size distribution as measured by a pore size distribution span 90 value, which pore size distribution span 90 value is calculated as $[(PD90 - PD10)/PD50]$, whereas PD10 is the pore diameter at 10% cumulative pore volume contribution, PD50 is the pore diameter at 50% cumulative pore volume contribution, and PD90 is the pore diameter at 90% cumulative pore volume contribution, all based on mercury intrusion data, of at least 2.8;
and
polymeric material covalently bonded to surfaces of said silica particles.

2. The adsorbents of claim 1, wherein said silica particles have a median particle size of less than 150 μm , preferably less than 100 μm , more preferred 10 to 50 μm , in particular 30 μm .

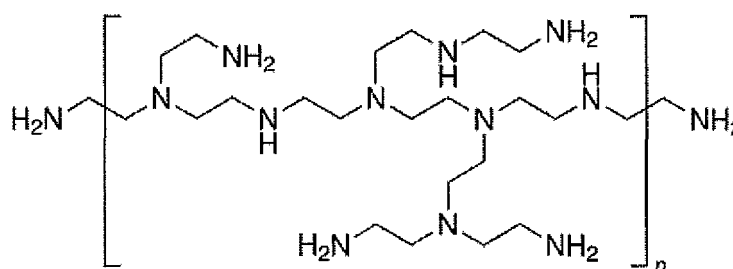
3. The adsorbents of any one of claims 1 or 2, wherein said silica particles have a median pore size, measured by mercury intrusion using an Autopore® IV 9520, available from Micromeritics Instrument Corporation, of less than 200 nm (2000 Å), preferably less than 100 nm (1000 Å), more preferred 10 nm to 80 nm (100 to about 800 Å), still more preferred 15 nm to 25 nm (150 to 250 Å), in particular 20 nm (200 Å).

4. The adsorbents of any one of claims 1 to 3, wherein said silica particles have a pore size distribution, which is the relative abundance of each pore size in a representative volume of porous inorganic particles, of from 3 to 500 nm (30 to 5000 Å), preferably 5 to 300 nm (50 to 3000 Å).

5. The adsorbents of any one of claims 1 to 4, wherein said silica particles have a BET particle surface area of at least 100 m^2/g , preferably at least 300 m^2/g , more preferred at least 500 m^2/g , still more preferred 600 to 800 m^2/g , in

particular 700 m²/g.

6. The adsorbents of any one of claims 1 to 5, wherein said silica particles have a pore volume, as measured by mercury intrusion, of at least 0.25 ml/g, preferably at least 0.50 ml/g, more preferred at least 1.00 ml/g, still more preferred at least 1.50 ml/g, in particular 1.77 ml/g.
7. The adsorbents of any one of claims 1 to 6, wherein said silica particles have a pore size distribution span 90 value of at least 3.0, more preferred 5.0 to 10.0, still more preferred 7.5 to 8.5, in particular 7.8.
8. The adsorbents of any one of claims 1 to 7, further comprising at least one bifunctional moiety extending from said surfaces of said silica particles, each of said at least one bifunctional moiety comprising (i) one or more functional groups capable of bonding to said surfaces, and either (iia) said polymeric material, or (iib) one or more reactive groups capable of bonding to said polymeric material.
9. The adsorbents of claim 8, wherein said at least one bifunctional moiety comprises an epoxy silane.
10. The adsorbents of any one of claims 8 or 9, wherein said at least one bifunctional moiety comprises (3-glycidoxypentyl)trimethoxysilane.
11. The adsorbents of any one of claims 1 to 10, wherein said polymeric material is covalently bonded to said surfaces via a covalent bond linkage consisting of one or more atoms selected from the group consisting of C, O, Si and N.
12. The adsorbents of any one of claims 1 to 11, wherein said polymeric material comprises a polyelectrolyte, preferably said polymeric material comprises polyethyleneimine, polyallylamine, polyvinyl pyridine, polydiallyldimethylammonium chloride (pDADMAC), or a copolymer containing one or more cationic or cationic-forming functional groups, in particular polyethyleneimine.
13. The adsorbents of any one of claims 1 to 12, wherein said polymeric material comprises trimethoxysilyl-polyethyleneimine.
14. The adsorbents of any one of claims 12 or 13, wherein said polyethyleneimine has (i) a weight average molecular weight, Mw, and (ii) a number average molecular weight, Mn, each of which is less than 5000, preferably (i) a weight average molecular weight, Mw, and (ii) a number average molecular weight, Mn, each of which ranges from 500 to 2500, more preferred (i) a weight average molecular weight, Mw, ranging from 800 to 2000, and (ii) a number average molecular weight, Mn, ranging from 600 to 1800.
15. The adsorbents of any one of claims 12 to 14, wherein said polyethyleneimine has a structure:



prior to being covalently bonded to said surfaces, and n is a number greater than or equal to 1.

16. The adsorbents of any one of claims 1 to 12, wherein said polymeric material comprises an anionic polyelectrolyte.
17. The adsorbents of any one of claims 1 to 12 and 16, wherein the polymeric material comprises polyacrylic acid, polymethacrylic acid, polystyrene sulfonic acid, nucleic acid, or a copolymer containing one or more anionic or anionic-forming functional groups, preferably polyacrylic acid, polymethacrylic acid, or a combination thereof, wherein said polyacrylic acid or said polymethacrylic acid preferably has a weight average molecular weight, M_w , greater than 50,000, more preferred 100,000 to 250,000.

18. The adsorbents of any one of claims 1 to 17, wherein said polymeric material is present in an amount representing up to 20.0 wt% of said adsorbents based on a total weight of said adsorbents, preferably 1.0 to 15.0 wt% of said adsorbents based on a total weight of said adsorbents.
19. The adsorbents of any one of claims 1 to 18, wherein less than 100% of said surfaces are bonded to said polymeric material.
20. The adsorbents of any one of claims 1 to 19 bound to one or more particular biological compounds, wherein the one or more particular biological compounds preferably comprise a protein, a polysaccharide, a lipid, a nucleic acid, a metabolites, a mammalian cell, mammalian cell debris, an antibody, a peptide, DNA, RNA, an endotoxin, a virus, a vaccine, an enzyme, or any combination thereof.
21. A method of making the adsorbents of any one of claims 1 to 19, said method comprising:
contacting the silica particles with one or more reactants under reaction conditions that result in the polymeric material covalently bonded to the surfaces of the silica particles.
22. The method of claim 21, wherein the one or more reactants comprise at least one bifunctional compound comprising (i) one or more functional groups capable of bonding the compound to the surfaces of the silica particles, and either (iia) the polymeric material, or (iib) one or more reactive groups capable of bonding to the polymeric material.
23. The method of any one of claims 21 and 22, wherein said contacting step utilizes a reaction mixture free of organic solvent.
24. Use of adsorbents as defined in any of claims 1 to 19 as a bioprocessing clarification agent.
25. Use of the adsorbents according to claim 24, wherein the adsorbents is added to a bioprocessing vessel containing one or more biomolecules, the one or more biomolecules preferably comprising a protein, a polysaccharide, a lipid, a nucleic acid, a metabolites, a mammalian cell, mammalian cell debris, an antibody, a peptide, DNA, RNA, an endotoxin, a virus, a vaccine, an enzyme, or any combination thereof.
26. Use of the adsorbents according to any one of claims 24 or 25, wherein the adsorbents bind to one or more biomolecules that come into contact with the adsorbents.
27. Use of the adsorbents according to any one of claims 24 to 26, wherein the adsorbents is added to a bioprocessing vessel containing at least one of: host cells and cell debris; antibodies, proteins and peptides; nucleic acids, such as DNA and RNA; endotoxins; viruses; vaccines.
28. Use of the adsorbents according to any one of claims 24 to 27, wherein following a binding step in which the adsorbents binds to one or more biomolecules that come into contact with the adsorbents, separating the adsorbents bound to the one or more biomolecules from other components of a bioprocessing procedure.

Patentansprüche

1. Adsorbenzien, umfassend:

Silicateilchen mit einer breiten Porengrößenverteilung, wie durch einen Span-90-Wert der Porengrößenverteilung gemessen, von mindestens 2,8, wobei der Span-90-Wert der Porengrößenverteilung als $[(PD90 - PD10)/PD50]$ berechnet wird, wobei PD10 der Porendurchmesser bei 10 % kumulativem Porenvolumenbeitrag ist, PD50 der Porendurchmesser bei 50 % kumulativem Porenvolumenbeitrag ist, und PD90 der Porendurchmesser bei 90 % kumulativem Porenvolumenbeitrag ist, alle basierend auf Quecksilberintrusionsdaten; und
Polymermaterial, das kovalent an Oberflächen der Silicateilchen gebunden ist.

2. Adsorbenzien nach Anspruch 1, wobei die Silicateilchen eine mittlere Teilchengröße von weniger als 150 μm , vorzugsweise weniger als 100 μm , mehr bevorzugt 10 bis 50 μm , insbesondere 30 μm , aufweisen.
3. Adsorbenzien nach einem der Ansprüche 1 oder 2, wobei die Silicateilchen eine mittlere Porengröße, gemessen

durch Quecksilberintrusion unter Verwendung eines Autopore® IV 9520, erhältlich von Micromeritics Instrument Corporation, von weniger als 200 nm (2000 Å), vorzugsweise weniger als 100 nm (1000 Å), mehr bevorzugt 10 nm bis 80 nm (100 bis etwa 800 Å), noch mehr bevorzugt 15 nm bis 25 nm (150 bis 250 Å), insbesondere 20 nm (200 Å), aufweisen.

- 5 4. Adsorbenzien nach einem der Ansprüche 1 bis 3, wobei die Silicateilchen eine Porengrößenverteilung, die die relative Häufigkeit jeder Porengröße in einem repräsentativen Volumen poröser anorganischer Teilchen ist, von 3 bis 500 nm (30 bis 5000 Å), vorzugsweise 5 bis 300 nm (50 bis 3000 Å) aufweisen.
- 10 5. Adsorbenzien nach einem der Ansprüche 1 bis 4, wobei die Silicateilchen eine BET-Teilchenoberfläche von mindestens 100 m²/g, vorzugsweise mindestens 300 m²/g, mehr bevorzugt mindestens 500 m²/g, noch mehr bevorzugt 600 bis 800 m²/g, insbesondere 700 m²/g, aufweisen.
- 15 6. Adsorbenzien nach einem der Ansprüche 1 bis 5, wobei die Silicateilchen ein Porenvolumen, wie durch Quecksilberintrusion gemessen, von mindestens 0,25 ml/g, vorzugsweise mindestens 0,50 ml/g, mehr bevorzugt mindestens 1,00 ml/g, noch mehr bevorzugt mindestens 1,50 ml/g, insbesondere 1,77 ml/g, aufweisen.
- 20 7. Adsorbenzien nach einem der Ansprüche 1 bis 6, wobei die Silicateilchen einen Span-90-Wert der Porengrößenverteilung von mindestens 3,0, mehr bevorzugt 5,0 bis 10,0, noch mehr bevorzugt 7,5 bis 8,5, insbesondere 7,8, aufweisen.
- 25 8. Adsorbenzien nach einem der Ansprüche 1 bis 7, ferner umfassend mindestens einen bifunktionellen Molekülteil, der sich von den Oberflächen der Silicateilchen erstreckt, wobei der mindestens eine bifunktionelle Molekülteil jeweils (i) eine oder mehrere funktionelle Gruppen, die fähig sind, an die Oberflächen zu binden, und entweder (iia) das Polymermaterial oder (iib) eine oder mehrere reaktive Gruppen, die fähig sind, an das Polymermaterial zu binden, umfasst.
- 30 9. Adsorbenzien nach Anspruch 8, wobei der mindestens eine bifunktionelle Molekülteil ein Epoxysilan umfasst.
- 35 10. Adsorbenzien nach einem der Ansprüche 8 oder 9, wobei der mindestens eine bifunktionelle Molekülteil (3-Glycidoxypropyl)trimethoxysilan umfasst.
- 40 11. Adsorbenzien nach einem der Ansprüche 1 bis 10, wobei das Polymermaterial über eine kovalente Bindungsverknüpfung, die aus einem oder mehreren Atomen besteht, ausgewählt aus der Gruppe bestehend aus C, O, Si und N, kovalent an die Oberflächen gebunden ist.
- 45 12. Adsorbenzien nach einem der Ansprüche 1 bis 11, wobei das Polymermaterial einen Polyelektrolyten umfasst, wobei das Polymermaterial vorzugsweise Polyethylenimin, Polyallylamin, Polyvinylpyridin, Polydiallyldimethylammoniumchlorid (pDADMAC) oder ein Copolymer, das eine oder mehrere kationische oder kationenbildende funktionelle Gruppen enthält, insbesondere Polyethylenimin, umfasst.
- 50 13. Adsorbenzien nach einem der Ansprüche 1 bis 12, wobei das Polymermaterial Trimethoxysilyl-Polyethylenimin umfasst.
- 55 14. Adsorbenzien nach einem der Ansprüche 12 oder 13, wobei das Polyethylenimin (i) ein gewichtsmittleres Molekulargewicht Mw und (ii) ein zahlenmittleres Molekulargewicht Mn, von denen jedes weniger als 5000 beträgt, vorzugsweise (i) ein gewichtsmittleres Molekulargewicht Mw und (ii) ein zahlenmittleres Molekulargewicht Mn, von denen jedes im Bereich von 500 bis 2500 liegt, mehr bevorzugt (i) ein gewichtsmittleres Molekulargewicht Mw im Bereich von 800 bis 2000 und (ii) ein zahlenmittleres Molekulargewicht Mn im Bereich von 600 bis 1800 aufweist.
15. Adsorbenzien nach einem der Ansprüche 12 bis 14, wobei das Polyethylenimin eine folgende Struktur aufweist:

technikgefäß gegeben werden, das mindestens eines enthält von: Wirtszellen und Zelltrümmern; Antikörpern, Proteinen und Peptiden; Nukleinsäuren wie DNA und RNA; Endotoxinen; Viren; Impfstoffen.

28. Verwendung der Adsorbenzien nach einem der Ansprüche 24 bis 27, wobei, einem Bindungsschritt folgend, bei dem die Adsorbenzien an ein oder mehrere Biomoleküle binden, die mit den Adsorbenzien in Kontakt kommen, die an das eine oder die mehreren Biomoleküle gebundenen Adsorbenzien von anderen Komponenten einer Bioprozesstechnikprozedur getrennt werden.

Revendications

1. Adsorbants comprenant :

des particules de silice ayant une large distribution volumique de pores telle que mesurée par une valeur d'étendue 90 de distribution volumique de pores, laquelle valeur d'étendue 90 de distribution volumique de pores est calculée en tant que $[(PD90 - PD10)/PD50]$, en considérant que PD10 est le diamètre de pore à une contribution cumulative de volume de pore de 10 %, PD50 est le diamètre de pore à une contribution cumulative de volume de pore de 50 %, et PD90 est le diamètre de pore à une contribution cumulative de volume de pore de 90 %, tous basés sur des données d'intrusion de mercure, d'au moins 2,8 ;

et

un matériau polymère lié par covalence à des surfaces desdites particules de silice.

2. Adsorbants selon la revendication 1, dans lesquels lesdites particules de silice ont une taille médiane de particule inférieure à 150 μm , de préférence inférieure à 100 μm , plus préférablement 10 à 50 μm , en particulier de 30 μm .

3. Adsorbants selon l'une quelconque des revendications 1 ou 2, dans lesquels lesdites particules de silice ont une taille médiane de pore, mesurée par intrusion de mercure en utilisant un Autopore® IV 9520, disponible auprès de Micromeritics Instrument Corporation, inférieure à 200 nm (2000 Å), de préférence inférieure à 100 nm (1000 Å), plus préférablement de 10 nm à 80 nm (100 à environ 800 Å), encore plus préférablement de 15 nm à 25 nm (150 à 250 Å), en particulier de 20 nm (200 Å).

4. Adsorbants selon l'une quelconque des revendications 1 à 3, dans lesquels lesdites particules de silice ont une distribution volumique de pores, qui est l'abondance relative de chaque taille de pore dans un volume représentatif de particules inorganiques poreuses, allant de 3 à 500 nm (30 à 5000 Å), de préférence 5 à 300 nm (50 à 3000 Å).

5. Adsorbants selon l'une quelconque des revendications 1 à 4, dans lesquels lesdites particules de silice ont une surface spécifique de particule BET d'au moins 100 m^2/g , de préférence au moins 300 m^2/g , plus préférablement au moins 500 m^2/g , encore plus préférablement 600 à 800 m^2/g , en particulier de 700 m^2/g .

6. Adsorbants selon l'une quelconque des revendications 1 à 5, dans lesquels lesdites particules de silice ont un volume de pore, tel que mesuré par intrusion de mercure, d'au moins 0,25 ml/g , de préférence au moins 0,50 ml/g , plus préférablement au moins 1,00 ml/g , encore plus préférablement au moins 1,50 ml/g , en particulier de 1,77 ml/g .

7. Adsorbants selon l'une quelconque des revendications 1 à 6, dans lesquels lesdites particules de silice ont une valeur d'étendue 90 de distribution volumique de pores d'au moins 3,0, plus préférablement 5,0 à 10,0, encore plus préférablement 7,5 à 8,5, en particulier de 7,8.

8. Adsorbants selon l'une quelconque des revendications 1 à 7, comprenant en outre au moins un fragment bifonctionnel s'étendant à partir desdites surfaces desdites particules de silice, chacun dudit au moins un fragment bifonctionnel comprenant (i) un ou plusieurs groupes fonctionnels capables de se lier auxdites surfaces, et soit (iia) ledit matériau polymère, soit (iib) un ou plusieurs groupes réactifs capables de se lier audit matériau polymère.

9. Adsorbants selon la revendication 8, dans lesquels ledit au moins un fragment bifonctionnel comprend un époxy-silane.

10. Adsorbants selon l'une quelconque des revendications 8 ou 9, dans lesquels ledit au moins un fragment bifonctionnel comprend du (3-glycidioxypropyl)triméthoxysilane.

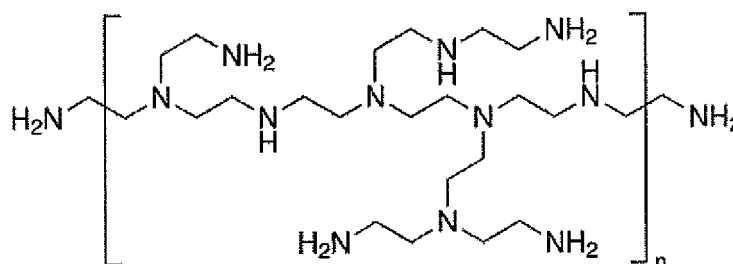
11. Adsorbants selon l'une quelconque des revendications 1 à 10, dans lesquels ledit matériau polymère est lié par covalence auxdites surfaces par l'intermédiaire d'une liaison covalente constituée d'un ou plusieurs atomes choisis dans le groupe constitué de C, O, Si et N.

12. Adsorbants selon l'une quelconque des revendications 1 à 11, dans lesquels ledit matériau polymère comprend un polyélectrolyte, de préférence ledit matériau polymère comprend polyéthylène-imine, polyallylamine, polyvinyl-pyridine, chlorure de polydiallyldiméthylammonium (pDADMAC), ou un copolymère contenant un ou plusieurs groupes fonctionnels cationiques ou formant des cations, en particulier polyéthylène-imine.

13. Adsorbants selon l'une quelconque des revendications 1 à 12, dans lesquels ledit matériau polymère comprend une triméthoxysilyl-polyéthylène-imine.

14. Adsorbants selon l'une quelconque des revendications 12 ou 13, dans lesquels ladite polyéthylène-imine a (i) une masse moléculaire moyenne en poids, Mw, et (ii) une masse moléculaire moyenne en nombre, Mn, dont chacune est inférieure à 5000, de préférence (i) une masse moléculaire moyenne en poids, Mw, et (ii) une masse moléculaire moyenne en nombre, Mn, dont chacune va de 500 à 2500, plus préférablement (i) une masse moléculaire moyenne en poids, Mw, allant de 800 à 2000, et (ii) une masse moléculaire moyenne en nombre, Mn, allant de 600 à 1800.

- 15.** Adsorbants selon l'une quelconque des revendications 12 à 14, dans lesquels ladite polyéthylène-imine a une structure :



avant d'être liée par covalence auxdites surfaces, et n est un nombre supérieur ou égal à 1.

- 16.** Adsorbants selon l'une quelconque des revendications 1 à 12, dans lesquels ledit matériau polymère comprend un polyélectrolyte anionique.

17. Adsorbants selon l'une quelconque des revendications 1 à 12 et 16, dans lesquels le matériau polymère comprend acide polyacrylique, acide polyméthacrylique, acide polystyrène-sulfonique, acide nucléique, ou un copolymère contenant un ou plusieurs groupes fonctionnels anioniques ou formant des anions, de préférence acide polyacrylique, acide polyméthacrylique, ou une combinaison de ceux-ci, dans lesquels ledit acide polyacrylique ou ledit acide polyméthacrylique a de préférence une masse moléculaire moyenne en poids, M_w , supérieure à 50 000, plus préférablement de 100 000 à 250 000.

- 18.** Adsorbants selon l'une quelconque des revendications 1 à 17, dans lesquels ledit matériau polymère est présent en une quantité représentant jusqu'à 20,0 % en poids desdits adsorbants sur la base d'un poids total desdits adsorbants, de préférence 1,0 à 15,0 % en poids desdits adsorbants sur la base d'un poids total desdits adsorbants.

- 19.** Adsorbants selon l'une quelconque des revendications 1 à 18, dans lesquels moins de 100 % desdites surfaces sont liées audit matériau polymère.

20. Adsorbants selon l'une quelconque des revendications 1 à 19 liés à un ou plusieurs composés biologiques particuliers, dans lesquels le ou les composés biologiques particuliers comprennent de préférence une protéine, un polysaccharide, un lipide, un acide nucléique, un métabolite, une cellule mammalienne, des débris de cellule mammalienne, un anticorps, un peptide, de l'ADN, de l'ARN, une endotoxine, un virus, un vaccin, une enzyme, ou n'importe quelle combinaison de ceux-ci.

- 21.** Procédé de fabrication des adsorbants selon l'une quelconque des revendications 1 à 19, ledit procédé comprenant : la mise en contact des particules de silice avec un ou plusieurs réactifs dans des conditions de réaction qui résultent

en le matériau polymère lié par covalence aux surfaces des particules de silice.

- 5 **22.** Procédé selon la revendication 21, dans lequel le ou les réactifs comprennent au moins un composé bifonctionnel comprenant (i) un ou plusieurs groupes fonctionnels capables de lier le composé aux surfaces des particules de silice, et soit (iia) le matériau polymère, soit (iib) un ou plusieurs groupes réactifs capables de se lier au matériau polymère.
- 10 **23.** Procédé selon l'une quelconque des revendications 21 et 22, dans lequel ladite étape de mise en contact utilise un mélange réactionnel exempt de solvant organique.
- 15 **24.** Utilisation d'adsorbants tels que définis dans l'une quelconque des revendications 1 à 19 en tant qu'agent de clarification de biotraitement.
- 20 **25.** Utilisation des adsorbants selon la revendication 24, dans laquelle les adsorbants sont ajoutés à un récipient de biotraitement contenant une ou plusieurs biomolécules, la ou les biomolécules comprenant de préférence une protéine, un polysaccharide, un lipide, un acide nucléique, un métabolite, une cellule mammalienne, des débris de cellule mammalienne, un anticorps, un peptide, de l'ADN, de l'ARN, une endotoxine, un virus, un vaccin, une enzyme, ou n'importe quelle combinaison de ceux-ci.
- 25 **26.** Utilisation des adsorbants selon l'une quelconque des revendications 24 ou 25, dans laquelle les adsorbants se lient à une ou plusieurs biomolécules qui viennent en contact avec les adsorbants.
- 30 **27.** Utilisation des adsorbants selon l'une quelconque des revendications 24 à 26, dans laquelle les adsorbants sont ajoutés à un récipient de biotraitement contenant au moins l'un parmi : des cellules hôtes et débris cellulaires ; des anticorps, protéines et peptides ; des acides nucléiques, tels qu'ADN et ARN ; des endotoxines ; des virus ; des vaccins.
- 35 **28.** Utilisation des adsorbants selon l'une quelconque des revendications 24 à 27, dans laquelle à la suite d'une étape de liaison dans laquelle les adsorbants se lient à une ou plusieurs biomolécules qui viennent en contact avec les adsorbants, on sépare les adsorbants liés à la ou aux biomolécules par rapport à d'autres composants d'une procédure de biotraitement.
- 40
- 45
- 50
- 55

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO 2010043700 A [0007]
- WO 2010043703 A [0007]
- US 2014367338 A [0007]
- US 4576927 A [0007]
- WO 2011012302 A [0007]
- WO 2002074791 A [0020]
- US 5451660 A [0020]
- US 4895806 A [0021]

Non-patent literature cited in the description

- **MINOW B. et al.** *BioProcess Int.*, 01 April 2014 [0006]
- **N. AZIZI ; M. R. SAIDI.** *Org. Lett.*, 2005, vol. 7, 3649-3651 [0069]