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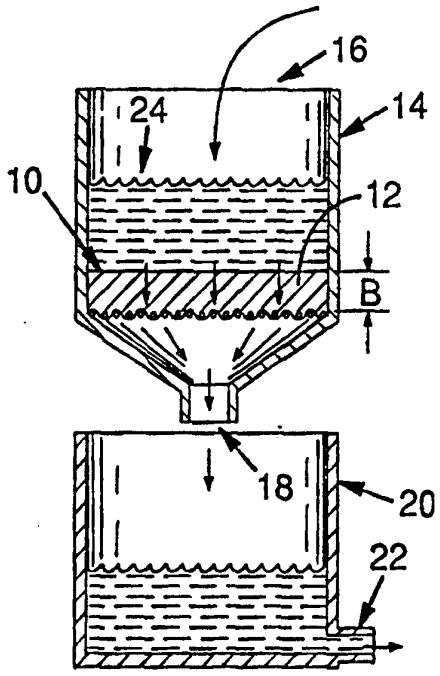
(54) Title: COMPOSITE BIOLOGICAL-MOLECULE-ACCRETION MATERIAL

(57) Abstract

A composite biological-molecule-accretion material (10) is described for extracting desired target biological molecules from aqueous solution (24) and substantially permanently binding to those molecules. The material (10) includes a first component (10a) designed with a binding affinity for proteins and peptides in such solution, and a second component (10b) designed with a binding affinity for nucleic acids in such solution. The material (10) is capable of being formed in a body (12) for passing such solution through it, and such passing has the effect of removing the proteins, peptides and nucleic acids from such solution and binding them to the body. That binding is substantially permanent so that subsequent passage of additional aqueous solution will not unbind substantially the accreted proteins, peptides and nucleic acids. The first component (10a) may include dextran-coated ($10a_2$) activated charcoal particles ($10a_1$), and the second component may include an anion-exchange resin and a cation-exchange resin. Preferred relative volumes of both components are 1:1. Also described is a method for extracting desired target biological molecules from aqueous solution and substantially permanently binding them to a solid, and a method of filtering target biological molecules out of water.



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COMPOSITE BIOLOGICAL-MOLECULE-ACCRETION MATERIAL

This is a continuation-in-part of priority U.S. Patent Application Serial No. 08/106,415 filed 13 August 1993.

Background and Summary of the Invention

The present invention relates generally to filters and other devices for removing undesired chemicals from solution, and more particularly to a composite biological-molecule-accretion material for extracting desired target biological molecules from aqueous solution and substantially permanently binding to those molecules.

There have been several conventional proposals for dealing with removal of unwanted chemicals from solution. However, none has proven adequate for dealing with removal and ultimate disposal of certain chemically labeled molecules regularly used in connection with basic and applied biological research. Those molecules will be referred to in the present application as target molecules, and a list of them is provided below:

Target Biological Molecules

1. peptides;
2. polypeptides;
3. RNA;
4. DNA;
5. enzymes;
6. nucleic acids;
7. proteins;
8. biopolymers; and
9. amino acids.

The essential problem for laboratories performing research with such molecules is that present environmental regulations no longer allow for simply pouring them down the drain after they are chemically labeled according to known procedures. Examples of such labeling involve using radioactivity or fluorescence to label a desired molecule. While radio-labeled molecules, sometimes referred to as probes, will be referred to throughout, it should be understood that the to-be-described invention is usable for removing any

chemically labeled target molecule from aqueous solution and substantially permanently binding to it.

Basic and applied biological research can also result in waste products characterizable as mixtures of toxic organic solutions and radio labeled target molecules in aqueous solutions. Such mixtures of solutions will be referred to herein as "mixed waste". The to-be-described invention is also usable for removing such radio labeled target molecules from such mixed waste.

Nowhere has there been shown or suggested to provide a composite biological-molecule-accretion material for (1) extracting desired target biological molecules from either aqueous solution or mixed waste, and (2) substantially permanently binding to those molecules. Accordingly, it is a principal object of the present invention to provide such a material that overcomes the drawbacks of prior-art materials.

Another object is to provide such a material that provides for such removal and binding to a relatively wide variety of molecules such as the target molecules listed above.

Yet another object is to provide such a material that has a relatively long working life.

Another important object of the invention is to provide such a material that can be readily and easily disposed of after its working life is over.

It is also an object of the invention to provide such a material that can be easily and cost-effectively manufactured.

In brief summary, one aspect of the invention includes a composite biological-molecule-accretion material for extracting (or removing) desired target biological molecules from aqueous solution and substantially permanently binding to those molecules. The material includes a first component designed with a binding affinity for proteins and peptides in such solution, and a second component designed with a binding affinity for nucleic acids in such solution. The material is capable of being formed in a body for passing such solution through it, and such passing has the effect of removing the proteins, peptides and nucleic acids from such solution and binding them to the body. That binding is substantially permanent so that subsequent passage of additional aqueous solution

will not unbind substantially the accreted proteins, peptides and nucleic acids from the body.

Preferably the first component consists essentially of dextran-coated activated charcoal particles, and the second component consists essentially of an anion-exchange resin and a cation-exchange resin. The preferred relative volumes of both components are 1:1, and the relative volumes of the two ion-exchange resins is 1:1. As a result, relative volumes for the composition of the preferred version of the material is 2 parts dextran-coated charcoal particles to 1 part anion-exchange resin to 1 part cation-exchange resin.

Another aspect of the present invention includes a method of filtering target biological molecules out of water. The method includes the step of forming a filter bed by soaking a mixture of dextran-coated, activated charcoal in water for a preselected time period, adding to the mixture an ion-exchange resin with a preselected charge-sensitivity, drying the mixture, and rehydrating the mixture. The method also includes the step of placing the filter bed into a water-filtration apparatus with an input and an output, and passing water containing the target biological molecules through the filtration apparatus to capture substantially the target biological molecules in the filter bed.

These and other objects and advantages of the invention will be more clearly understood from a consideration of the accompanying drawings and the following description of the preferred embodiment.

Brief Description of the Drawings

Fig. 1 is an isometric view of the accretion material of the present invention after being formed in a body (or filter bed) and being placed into conventional water-filtration apparatus.

Fig. 2 is a side-sectional view through line 2-2 of Fig. 1 with the accretion material being formed in a body, and with solid lines indicating an unswelled condition of the material, and dashed lines indicating a swelled condition of the same.

Fig. 3 is a fragmentary, greatly enlarged, schematic view of the unswelled body of accretion material shown in Fig. 2.

Fig. 4 is a fragmentary, greatly enlarged, schematic view of the swelled body of accretion material shown in Fig. 2.

Fig. 5 is a greatly enlarged view of unswelled charcoal particles forming part of the accretion material.

Fig. 6 shows the charcoal particles of Fig. 5 after swelling.

Fig. 7 shows the charcoal particles of Fig. 6 after being coated with dextran substance.

Fig. 8 shows the charcoal particles of Fig. 7 being mixed with anion- and cation-exchange resins, with the resins being indicated schematically by encircled +'s and -'s.

Fig. 9 shows the charcoal particles of Fig. 8 after being mixed with the resins.

Fig. 10 is like Fig. 2 only that it shows the swelled condition of the body of accretion material in solid lines, and shows introduction into the conventional water-filtration apparatus of aqueous solution containing desired target biological molecules, with the solution passing through the body.

Detailed Description of the Preferred Embodiment

Referring generally to Figs. 1-4 and 10, applicant will describe generally how he proposes using the invented composite biological-molecule-accretion material, which material is indicated at 10 being made in accordance with its to-be-described preferred embodiment. After describing how the invented material is used, applicant will discuss details of its composition. Material 10 is formed in a body 12, with body 12 being placed in a conventional water-filtration apparatus 14. In its place in apparatus 14, body 12 will allow a to-be-described aqueous solution to pass through it, with such passing having the effect of removing certain target molecules such as proteins, peptides and nucleic acids from such solution and binding them to body 12. The binding is substantially permanent in that subsequent passage of additional aqueous solution will not unbind substantially the accreted proteins, peptides and nucleic acids from the body. As will be shown, material 10 does not unbind substantially from the accreted proteins, peptides and nucleic acids even when subsequent passage of

additional aqueous solution includes relatively harsh components such as detergents.

Still referring to Figs. 1-4 and 10, conventional water-filtration apparatus 14 is constructed with an input 16 and an output 18, and includes a receptacle 20 for receiving effluent from output 18. Receptacle 20 is formed with a suitable port 22, which may be an aspiration port, for dispensing effluent from the receptacle. The port may be fitted with the usual control valve (undepicted).

It should also be understood that a suitable support screen (undepicted) may be provided within apparatus 14 and around body 12 to hold material 10 in place so that the to-be-described aqueous solution must pass through body 12 after being poured through input 16.

While undepicted, it should be understood that multiple stages of filtration are possible, if desired, to perform a backup function as a way of removing even more chemically-labeled target molecules from waste solutions. In other words, the invention may include an arrangement of two or more apparatuses, like apparatus 14, with each such apparatus having a body of material, an input, and output, each like those described above. Preferably, such arrangement would include a vertical stacking of such apparatuses, with the bottom apparatus being placed over a receptacle like receptacle 20. As used herein, a one-stage filtration system is one like that depicted in Fig. 1. A two-stage filtration system is one that has a vertical stack of two apparatuses, like apparatus 14, with the bottom apparatus being placed over a receptacle like receptacle 20. To stack such apparatuses, it is presently proposed to use suitable support structure for stabilizing the stack.

Refocusing on material 10, certain physical characteristics of accretion material 10 will also be described before describing its composition. Referring to Fig. 2, body 12 is shown in an unswelled condition with solid lines between bracketed area A, and in a swelled condition up to a dashed line between bracketed area B. The significance of the unswelled/swelled condition will be described. The unswelled condition of body 12 is also shown in a greatly enlarged, schematic view in Fig. 4. Similarly, Fig. 5 shows the swelled condition in a greatly enlarged, schematic view.

Referring to Figs. 5-9, a schematic presentation of a method of forming material 10 is shown. As will be described, material 10 includes a first component 10a (Fig. 7) designed with a binding affinity for proteins and peptides in aqueous solution such as solution 24 shown in to-be-described Fig. 10. Material 10 also includes a second component 10b (Figs. 8-9) designed with a binding affinity for nucleic acids in such solution.

From an overview, Figs. 5-7 show formation of first component 10a, and Figs. 8-9 show introduction and then mixing of second component 10b with first component 10a. The first component includes, and preferably consists essentially of, dextran-coated activated charcoal particles, which exhibit the requisite binding affinity for proteins and peptides. The second component includes, and preferably consists essentially of, an anion-exchange resin and a cation-exchange resin. Those resins exhibit the requisite binding affinity for nucleic acids. Material 10 is preferably formed of 1 part by volume of first component 10a and 1 part by volume of second component 10b. Second component 10b is preferably formed of 1 part by volume anion-exchange resin and 1 part by volume cation-exchange resin. Therefore, the preferred formulation of material 10, by volume, is 2 parts first component 10a to 1 part anion-exchange resin and 1 part cation-exchange resin. The total volume of ion exchange resin (both anion- and cation-exchange resins) in the preferred embodiment is about one-third to one-half of the volume of the dextran-coated activated charcoal. Also, suitable quantities of suitable filler material, such as course sand, may be added to increase flow rate through material 10.

With material 10 being formed into body 12, it may also be characterized as a target-biological-substance-retaining filter for use in a filter system to remove substantially and selectively desired target biological substances from an aqueous solution (e.g. solution 24 of Fig. 10). First component 10a may also be thought of as a filter-matrix component formed from first and second subcomponents. The first subcomponent is preferably activated charcoal particles, and the second component is a water-soluble, high-molecular-weight substance such as dextran. Second component 10b may also be thought of as a charge-carrier component distributed substantially in the filter-matrix component to

provide a plurality of charged sites therein. The filter-matrix component (first component 10a) and the charge-carrier component (second component 10b) are usable in a desired thickness (as body 12) in a filter system (apparatus 14) to provide a filter that defines paths through its thickness for allowing the aqueous solution (e.g. solution 24 of Fig. 10) to pass, and removes substantially the target biological substances by binding them to the charged sites.

Material 10 being formed as body 12 may also be characterized in yet another way as a filter bed for use in a filter system to remove target biological substances contained in an aqueous solution (e.g. solution 24 of Fig. 10). First component 10a may be characterized as means for forming a filter matrix that is porous substantially only to that portion of such solution that does not include the target biological substances. The filter-matrix-forming means includes first and second subcomponents, activated charcoal particles and a water-soluble, high-molecular-weight substance, respectively. The high-molecular-weight substance is preferably dextran, and it is mixed with the charcoal particles to coat them. Second component 10b may be characterized as a charge-carrier component distributed substantially in the filter-matrix-forming means to provide a plurality of charged sites therein. The filter-matrix-forming means and the charge-carrier component are usable in a desired thickness (i.e. body 12) in a filter system (i.e. apparatus 14) to provide a filter bed that defines paths through its thickness for allowing the aqueous solution (solution 24 of Fig. 10) to pass, and removes substantially the target biological substances by binding them to the charged sites.

Referring to Figs. 5-7, preparation of material 10 is shown schematically with first component 10a being formed by soaking a mixture of activated charcoal $10a_1$ and dextran $10a_2$ in water (undepicted) for a preselected time period. Figs. 5-6 show the swelling of charcoal $10a_1$ in water prior to addition of dextran $10a_2$. The presently preferred time period is about one hour. Next, referring to Figs. 8-9, second component 10b, taking the form of preselected anion- and cation-exchange resins (shown schematically as encircled +'s and -'s), is mixed with first component 10a. The resins forming second component 10b have a preselected charge-sensitivity, and preferred ones will be described below

in connection with example formulations. After mixing the first and second components, the mixture is aspirated to remove residual water and allowed to dry, and then rehydrated with a suitable amount of water so that it swells. Applicant has found that such rehydration promotes formation of the above-described filter matrix within body 12, and lessens the possibility that some target molecules will be missed when the first amount of the aqueous solution (e.g. solution 24 of Fig. 10) is passed through body 12.

EXAMPLE I

Material 10 was prepared using 45% activated charcoal such as that marketed under the trademark "NORIT A", 25% Whatman DE52 pre-swollen microgranular anion exchanger (diethylaminoethyl cellulose, catalog number 4057 050), 25% Rolm & Haas Co. Amberlite CG-50 (weakly acidic cation exchanger, carboxylic type, hydrogen form, wet mesh 100-200), and 5% course sand. An amount of that material having a wet volume of 100 ml was formed in a body like body 12 and placed in a filter structure like apparatus 14. 20μCi of ^{35}S-ATP in 800ml of Tris/Borate EDTA buffer was passed through the body of material 10. After subsequently passing 800ml of aqueous solution containing no radio-labeled biomolecules through the material, the total effluent contained only .37μCi of radioactive isotope, indicating a permanent binding efficiency of approximately 98% for ATP.

EXAMPLE II

Material 10 was prepared as in example I and placed in a filter structure like apparatus 14. Solutions containing 24μCi of ^{32}P-CTP DNA probe in 0.1% sodium dodecyl sulfate solution were passed through material 10. Despite the harsh character of a detergent like the sodium dodecyl sulfate solution, only .2μCi of the ^{32}P-CTP DNA probe passed through the material, indicating a permanent binding efficiency of greater than 99%.

EXAMPLE III

Material 10 was prepared as in example I and placed in a filter structure like apparatus 14. 49μCi of ^{32}P-labeled, 1450 base-pair DNA fragment in a 50% formamide solution was passed through the material. Analysis of the

effluent from apparatus 14 revealed .3μCi of radio-isotope passed through the material, indicating a permanent binding efficiency of greater than 99%.

EXAMPLE IV

Material 10 was prepared as in example I and placed in a filter structure like apparatus 14. A mixed waste solution of HPLC waste (40% acetonitrile/60% water - by volume) containing radiolabeled peptide was passed through the material. Specifically, 300-mL of the solution containing approximately 4.5μCi ^{125}I-βEP (β-endorphine) was passed through the material. Analysis of the effluent from apparatus 14 revealed less than 0.0004 μCi of radio-isotope passed through the material, indicating a permanent binding efficiency of greater than 99.99%. Over a 48-hour time period, water was intermittently passed through the material, and analysis of the effluent after such time period indicated no change in the 99.99+% permanent binding efficiency.

A prolonged use of material 10 in a one-stage filtration system was run over an 8-month period to remove ^{35}S radioactive nucleotides. Specifically, an amount of material 10 having a wet volume of 100 ml was formed in a body like body 12 and placed in a filter structure like apparatus 14. 58 units of aqueous solutions containing ^{35}S radioactive nucleotides were passed through the amount of material 10, and the average % of radioactive material absorbed by the amount of that material was 91.3%.

Additional, similar experiments involving radiolabeled proteins, peptides and nucleic acids revealed similar results, with approximately 98-99% of such molecules becoming substantially permanently bound to the accretion material as the aqueous solution containing such molecules was passed through an amount of the material formed into a body such as a filter bed. The additional experiments have involved solutions with ^{125}I-labeled peptides, ^{35}S-labeled amino-acid monomers. When using a body of material 10 having a wet volume of 100 ml, the time for passage through the body of aqueous solution containing target molecules ranged from about 10 minutes with aspiration, to overnight under gravity feed.

Material 10 worked as described above with acidic or basic aqueous solutions. It has also indicated a relatively long working life, i.e. an amount of

material 10 with a wet volume of 100 ml continues to exhibit the above binding efficiency of 98-99% after weekly use in a biological laboratory for approximately six months.

For situations where it is important to control the pH of solutions that are passed through material 10, buffer-like components may be added to material 10. It is presently proposed to use solid forms of acid- or base-leaching substances. For example, solid masses or nuggets of NaOH may be uniformly distributed in the material, using suitable mixing apparatus, to act as a buffer when acidic solutions are passed through material 10. Likewise, suitable weak acids may be used, in solid masses/nuggets, to act as a buffer when basic solutions are passed through material 10.

Operation and Preferred Method of Practicing

Using the above-described biological-molecule-accretion material 10, which is a solid, a method may be practiced for extracting desired target biological molecules from aqueous solution and substantially permanently binding them to that solid. The method includes the step of forming a solid with a binding affinity for such molecules. The forming step is preferably practiced by making the above-described biological-molecule-accretion material 10. The method also includes the steps of shaping the solid into a body and placing the body into a water-filtration apparatus with an input and an output, and passing aqueous solution containing such molecules through the filtration apparatus to remove them substantially from such solution, and substantially permanently bind them to the solid. Next, there is a step of repeating the passing step with additional aqueous solution and maintaining binding of the accreted proteins, peptides and nucleic acids to the solid.

Use of material 10 also involves a method of filtering target biological molecules out of water. That method includes a step of forming a filter bed by the substeps of soaking a mixture of dextran and activated charcoal in water for a preselected time period, adding to the mixture an ion-exchange resin with a preselected charge-sensitivity, drying the mixture, and then rehydrating the mixture. The second step involves placing the filter bed into a water-filtration apparatus with an input and an output, and the third step involves passing water

containing the target biological molecules through the filtration apparatus to capture substantially the target biological molecules in the filter bed.

It is presently proposed to maintain material 10 in a wet condition throughout its working life, although if the material were to dry out, a simple rehydration step could be performed as described above. Such rehydration was performed in connection with Example IV above and that amount of material 10 exhibited 99.99+% permanent binding efficiency.

When material 10 has exceeded its working life, it is dried by aspiration, removed from water-filtration apparatus 14, and disposed of as solid radioactive waste. Any effluent from aspirating may be poured down a drain if sufficiently low radioactivity is detected. It is presently contemplated that state and federal regulations do, or may soon, require extremely low or no radioactivity for effluent poured down a drain to a public water supply. With those regulations in mind, multiple-stage filtration systems utilizing material 10 may be used until a sufficiently low radioactivity is detected.

It should also be understood that material 10 could be tailored to be selective to certain types of molecules as opposed to being designed for removal of the entire class of target molecules listed above. For example, if one wanted to remove only those molecules with negative charges in aqueous solution (such as DNA compounds), then only a cation-exchange resin would be used as second component 10b.

The present invention achieves the above objects by providing a composite biological-molecule-accretion material for extracting desired target biological molecules from aqueous solution and substantially permanently binding to those molecules. That material overcomes the drawbacks of prior-art materials, and provides for such extraction/removal and binding to a relatively wide variety of molecules such as the target molecules listed above. The invented material has presently shown to have a relatively long working life, and when its working life is over the material can be disposed of readily and easily. Based on its preferred embodiment, the material can also be easily and cost-effectively manufactured.

Accordingly, while a preferred embodiment of the invention has been described herein, it is appreciated that modifications are possible that are within the scope of the invention.

I CLAIM:

1. A composite biological-molecule-accretion material for extracting desired target biological molecules from aqueous solution and substantially permanently binding to those molecules; comprising

a first component designed with a binding affinity for proteins and peptides in such solution;

a second component designed with a binding affinity for nucleic acids in such solution; and

wherein the material is capable of being formed into a body for passing such solution through it, with such passing having the effect of removing the proteins, peptides and nucleic acids from such solution and binding them to the body, and with the binding being substantially permanent so that subsequent passage of additional aqueous solution will not unbind substantially the accreted proteins, peptides and nucleic acids.

2. The material of claim 1 wherein the first component includes dextran-coated activated charcoal particles.

3. The material of claim 1 wherein the first component consists essentially of dextran-coated activated charcoal particles.

4. The material of claim 1 wherein the second component includes an anion-exchange resin and a cation-exchange resin.

5. The material of claim 1 wherein the second component consists essentially of an anion-exchange resin and a cation-exchange resin.

6. The material of claim 5 wherein the first component consists essentially of dextran-coated activated charcoal particles.

7. The material of claim 6 being formed of two parts by volume of the first component, one part by volume anion-exchange resin, and one part by volume cation-exchange resin.

8. The material of claim 1 wherein the body is capable of binding to DNA molecules contained in such solution and maintaining such binding to at least 95% of those molecules after subsequent passage of additional aqueous solution containing a detergent.

9. The material of claim 1 wherein the body is capable of binding to DNA molecules contained in such solution and maintaining such binding to at least 95% of those molecules after subsequent passage of additional aqueous solution containing 0.1% sodium dodecyl sulfate.

10. The material of claim 1 wherein the body is capable of binding to DNA molecules contained in such solution and maintaining such binding to at least 95% of those molecules after subsequent passage of additional aqueous solution containing 50% formamide solution.

11. The material of claim 1 wherein the body is capable of binding to peptides contained in mixed waste that includes a toxic organic solution and a radiolabeled peptide in aqueous solution, and maintaining such binding to at least 95% of those peptides after subsequent passage of aqueous solution.

12. The material of claim 1 wherein the body is capable of binding to an amount of ^{125}I-βEP contained in mixed waste that includes 40% acetonitrile/60% water by volume, and maintaining such binding to at least 95% of the amount of ^{125}I-βEP after subsequent passage of aqueous solution.

13. A target-biological-substance-retaining filter for use in a filter system to remove substantially and selectively desired target biological substances from an aqueous solution, comprising:

a filter-matrix component formed from first and second subcomponents, and with the second subcomponent being a water-soluble, high-molecular-weight substance;

a charge-carrier component distributed substantially in the filter-matrix component to provide a plurality of charged sites therein; and

with the filter-matrix component and the charge-carrier component being usable in a desired thickness in such filter system to provide a filter that defines paths through such thickness for allowing the aqueous solution to pass, and removes substantially the target biological substances by binding them to the charged sites.

14. A filter bed for use in a filter system to remove target biological substances contained in an aqueous solution, comprising:

means for forming a filter matrix that is porous substantially only to that portion of such solution that does not include the target biological substances, with the filter-matrix-forming means including first and second subcomponents, and with the second subcomponent being a water-soluble, high-molecular-weight substance;

a charge-carrier component distributed substantially in the filter-matrix-forming means to provide a plurality of charged sites therein; and

with the swelled filter-matrix-forming means and the charge-carrier component being usable in a desired thickness in such filter system to provide a filter bed that defines paths through its thickness for allowing such solution to pass, and removes substantially the target biological substances by binding them to the charged sites.

15. A method for extracting desired target biological molecules from aqueous solution and substantially permanently binding them to a solid, comprising:

forming a solid with a binding affinity for such molecules;

shaping the solid into a body and placing the body in a water-filtration apparatus with an input and an output;

passing aqueous solution containing such molecules through the filtration apparatus to remove them substantially from such solution, and substantially permanently bind them to the solid; and

repeating the passing step with additional aqueous solution and maintaining the binding of the accreted proteins, peptides and nucleic acids to the solid.

16. The method of claim 15, wherein the shaping step includes shaping the solid into plural bodies and positioning the bodies within the water-filtration apparatus in a sequence, and wherein the passing step includes passing aqueous solution containing such molecules through the sequence of bodies in the filtration apparatus.

17. A method of filtering target biological molecules out of water, comprising:

forming a filter bed by soaking a mixture of dextran and activated charcoal in water for a preselected time period, adding to the mixture an ion-exchange resin with a preselected charge-sensitivity, drying the mixture, and rehydrating the mixture;

placing the filter bed into a water-filtration apparatus with an input and an output; and

passing water containing the target biological molecules through the filtration apparatus to capture substantially the target biological molecules in the filter bed.

18. The method of claim 17, wherein the forming step includes forming plural filter beds and wherein the placing step includes positioning the filter beds in a sequence within the water-filtration apparatus, and wherein the passing step includes passing water containing the target molecules through the sequence of filter beds in the filtration apparatus.

FIG. 1

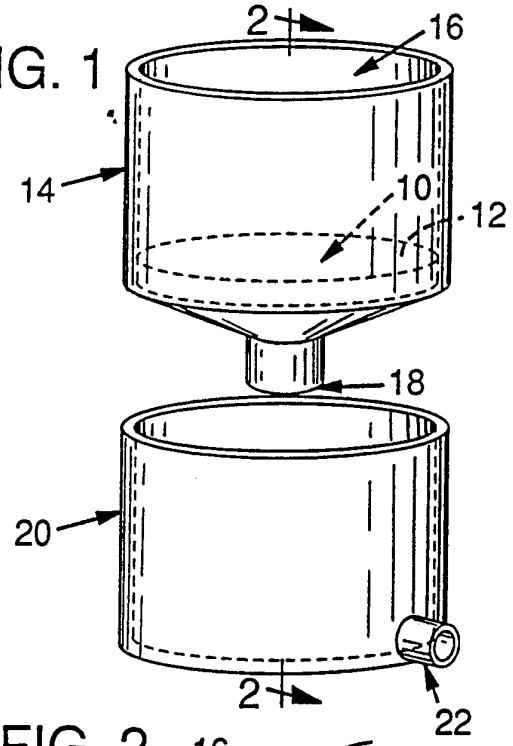


FIG. 3

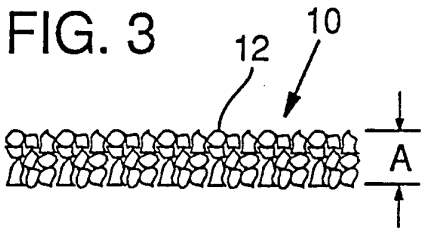


FIG. 4

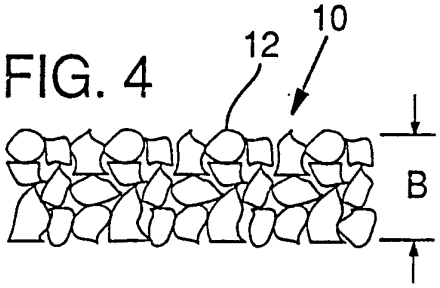


FIG. 2

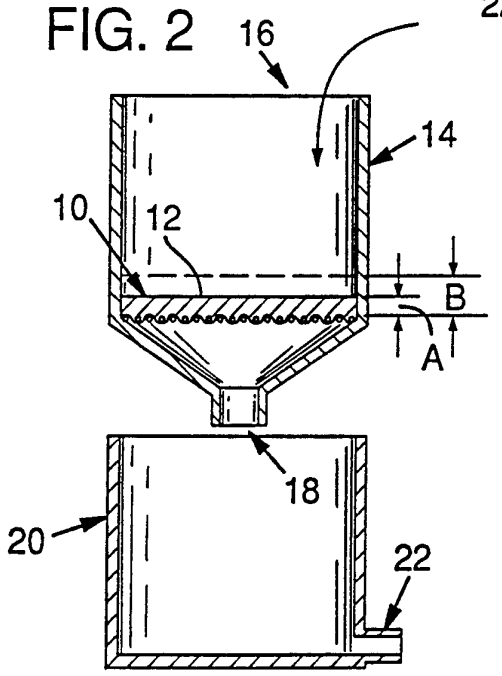


FIG. 10

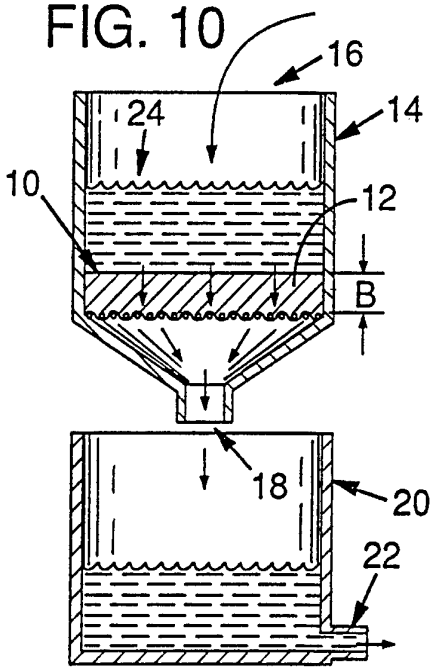


FIG. 5

FIG. 6

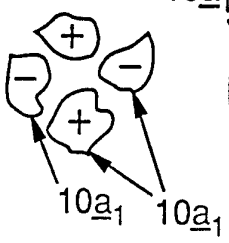


FIG. 7

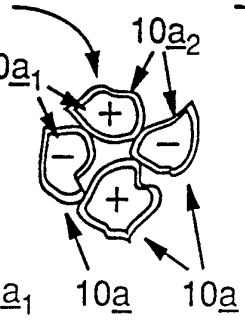


FIG. 8

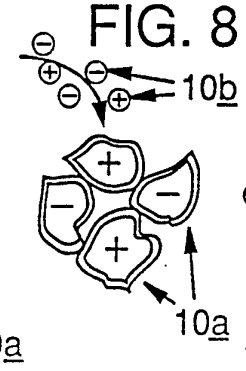
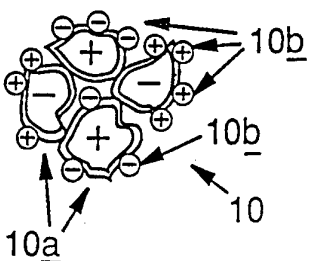


FIG. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/09187

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01J 20/20
US CL : 502/400,402,417
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 502/400,402,417

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,135,943 (MORISHITA ET AL), 23 JANUARY 1979, COL. 1, LINES 45-53; COL. 2, LINES 15-36	1-7
A	US, A, 4,897,467 (PORATH), 30 JANUARY 1990 , COL. 1, LINES 35-68	1
Y	US, A, 5,232,853 (SUGIYAMA ET AL), 03 AUGUST 1993, COL. 4, LINES 28-41	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
10 NOVEMBER 1994

Date of mailing of the international search report
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