

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
2 September 2004 (02.09.2004)

PCT

(10) International Publication Number  
**WO 2004/073843 A1**

(51) International Patent Classification<sup>7</sup>: **B01D 67/00**,  
69/10, 69/14, 69/12, 15/08, B01J 20/32, G01N 30/48

(21) International Application Number:  
PCT/CA2004/000120

(22) International Filing Date: 29 January 2004 (29.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/447,730 19 February 2003 (19.02.2003) US

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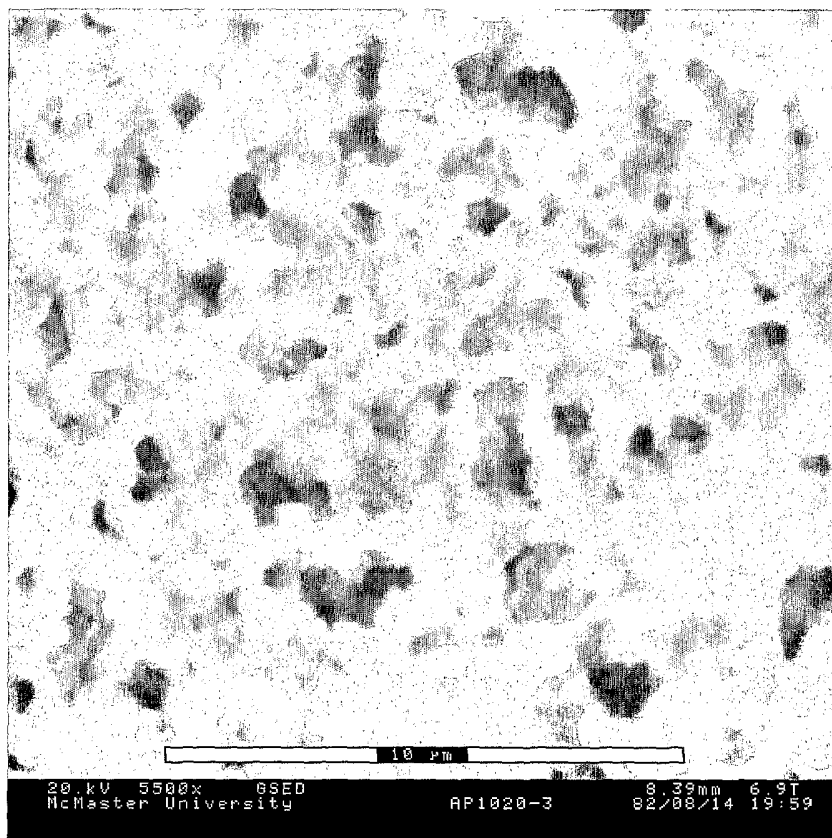
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,

[Continued on next page]

(54) Title: COMPOSITE MATERIALS COMPRISING SUPPORTED POROUS GELS



(57) Abstract: This invention relates to a composite material that comprises a support member that has a plurality of pores extending through the support member and, located in the pores of the support member, and filling the pores of the support member, a macroporous cross-linked gel. The invention also relates to a process for preparing the composite material described above, and to its use. The composite material is suitable, for example, for separation of substances, for example by filtration or adsorption, including chromatography, for use as a support in synthesis or for use as a support for cell growth.



CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— with international search report

**(84) Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## COMPOSITE MATERIALS COMPRISING SUPPORTED POROUS GELS

## FIELD OF THE INVENTION

This invention relates to composite materials that comprise supported macroporous cross-linked gels, and to their preparation and use. The composite materials are suitable, for example, for separation of substances, for example by filtration or adsorption, including chromatography, for use as a support in synthesis or for use as a support for cell growth.

## 10 BACKGROUND OF THE INVENTION

Composite materials and separation materials have been described in patent documents such as:

US Patent Nos. 4,224,415; 4,889,632; 4,923,610; 4,952,349; 5,160,627; 11/1992; 5,593,576; 5,599,453; 5,672,276; 15 5,723,601; 5,906,734; 6,045,697; 6,086,769; and 6,258,276;

International Patent Nos. EP 316,642; WO 00/12618; WO 00/50160; EP 316,642 B1; and EP 664,732 B1;

and in other publications, for example:

Liu, H.C. and Fried, J.R., Breakthrough of lysozyme through an affinity membrane of cellulose-Cibaron Blue. AIChE 20 Journal, vol. 40 (1994), p. 40-49.

Tennikov, M.B.; Gazdina, N.V.; Tennikova, T.B.; Svec, F., Effect of porous structure of macroporous polymer supports on resolution in high-performance membrane chromatography. 25 Journal of Chromatography A, vol. 798 (1998) p. 55-64.

Svec, F.; Jelinkova, M.; Votavova, E., Reactive macroporous membranes based on glycidyl methacrylate-ethylene

dimethacrylate copolymer for high-performance membrane chromatography. *Angew. Makromol. Chem.* Vol. 188 (1991) p. 167-176.

5 Tennikova, T.B.; Belenkii, B.G.; Svec, F., High-performance membrane chromatography. A novel method of protein separation. *J. Liquid Chromatography*, vol. 13 (1990) p. 63-70.

10 Tennikova, T.B.; Bleha, M.; Svec, F.; Almazova, T.V.; Belenkii, B.G.J., High-performance membrane chromatography of proteins, a novel method of protein separation. *Chromatography*, vol. 555 (1991) p. 97-107.

15 Tennikova, T.B.; Svec, F. High-performance membrane chromatography: highly efficient separation method for proteins in ion-exchange, hydrophobic interaction and reversed-phase modes. *J. Chromatography*, vol. 646 (1993) p. 279-288.

20 Viklund, C.; Svec, F.; Fréchet, J.M.J. Fast ion-exchange HPLC of proteins using porous poly(glycidyl methacrylate-co-ethylene dimethacrylate) monoliths grafted with poly(2-acrylamido-2-methyl-1-propanesulfonic acid). *Biotechnol. Progress*, vol. 13 (1997) p. 597-600.

Mika, A.M. and Childs, R.F. Calculation of the hydrodynamic permeability of gels and gel-filled microporous membranes, *Ind. Eng. Chem. Res.*, vol. 40 (2001), p. 1694-1705.

## 25 BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention provides a composite material that comprises a support member that has a plurality of pores extending through the support member and, located in the pores of the support member and essentially

filling the pores of the support member, a macroporous cross-linked gel. In some embodiments, the macroporous gel used is responsive to environmental conditions, providing a responsive composite material.

- 5 In another aspect, the invention provides a process for the separation of substances by means of the composite material described above.

In another aspect, the invention provides a process for solid phase chemical synthesis, wherein the composite  
10 material serves as the solid phase in the pores of which the chemical synthesis occurs.

In another aspect, the invention provides a process for growth of a microorganism or cell, wherein the composite material serves as a solid support in the pores of which the  
15 growth occurs.

In yet another aspect, the invention provides a process for preparing the composite material described above, the process comprising:

- a) introducing into the pores of the support member a  
20 solution or suspension containing

i) one or more monomers and one or more cross-linking agents that can combine to form a macroporous gel,  
or

- ii) one or more cross-linkable polymers and one or  
25 more cross-linking agents that can combine to form a macroporous gel,

b) reacting the monomers and the cross-linking agents or the polymers and the cross-linking agents to form a macroporous cross-linked gel that fills the pores of the support member.

The macroporous gel fills the pores of the support

5 laterally, i.e. substantially perpendicular to the direction of the flow through the composite material. By "fill" we mean that, in use, essentially all liquid that passes through the composite material must pass through the macroporous gel. A support member whose pores contain  
10 macroporous gel to such an amount that this condition is satisfied is regarded as filled. Provided that the condition is met that the liquid passes through the macroporous gel, it is not necessary that the void volume of the support member be completely occupied by the macroporous  
15 gel.

The porous support member, or host, may be hydrophilic or hydrophobic and can be, for example, in the form of a membrane, a chromatography bed, or a filtration bed. The support member provides the mechanical strength to support  
20 the macroporous gel. The macroporous gel provides a low resistance to hydraulic flow, enabling high flow rates to be achieved with low reductions in pressure across the composite material. The macroporous gel also provides the separating function of the composite material in  
25 chromatographic and filtration applications.

A gel is a cross-linked polymer network swollen in a liquid medium. The swelling liquid prevents the polymer network from collapsing and the network, in turn, retains the liquid.

30 Gels are typically obtained by polymerization of a monomer and a polyfunctional compound (a cross-linker), or by cross-

linking a cross-linkable polymer, in a solvent which is a good solvent for the formed polymer network and which swells the polymer network. The polymer chains in such a network can be assumed to be uniformly distributed throughout the whole volume of the network and the average distance between the chains, known as mesh size, is determined by the cross-linking density. As the concentration of the cross-linker is increased, the density of cross-links in the gel also increases, which leads to a smaller mesh size in the gel.

The smaller mesh size results in a higher resistance to the flow of liquids through the gel. As the concentration of the cross-linker is increased further, the constituents of the gel begin to aggregate, which produces regions of high polymer density and regions of low polymer density in the gel. Such gels exhibit what has been called microheterogeneity. This aggregation normally causes the gel to display a higher permeability to liquids, as the flow of liquids takes place primarily through the areas in the gel that have a lower polymer density. The low density areas of the gels are defined as draining regions while the higher density aggregates are called non-draining regions. As the concentration of the cross-linker is increased even further, leading to more cross-links, the gel can develop regions in which there is essentially no polymer. These regions are referred to as "macropores" in the present specification.

It is possible to compare the hydrodynamic (Darcy) permeability of a particular composite material of the invention with a reference material. The reference material is obtained by filling the pores of a support member identical with that of the composite material with a homogeneous gel of essentially the same chemical composition and the similar mass as the gel of the composite material, that is a gel composed of the same monomers formed in a good

solvent, but cross-linked only to such an extent that the gel remains homogeneous and aggregation into regions of high and low polymer density does not occur. Composite materials having macroporous gels display hydrodynamic (Darcy)

5 permeabilities that are at least one order of magnitude higher than those of the corresponding reference materials, and in some instances the permeabilities are more than two or even more than three orders of magnitude higher. In this specification, a composite material of the invention whose  
10 hydrodynamic (Darcy) permeability is at least an order of magnitude greater than that of the corresponding reference material is said to have a permeability ratio greater than 10.

The permeability ratio is closely related to the size of the  
15 macropores in the composite material. For size-exclusion separations such as ultrafiltration, the permeability ratio can be fairly close to 10. In other applications, for example adsorption, synthesis or cell growth, where larger macropores are used, the permeability ratio can reach, in  
20 some embodiments, values of 100 or greater, or even 1000 or greater. In some instances it is possible to calculate the hydrodynamic permeability of homogeneous gels, in accordance with the teachings of Mika A. M. and Childs R. F.

Calculation of the hydrodynamic permeability of gels and  
25 gel-filled microporous membranes, Ind. Eng. Chem. Res., vol. 40 (2001), p. 1694-1705, incorporated herein by reference. This depends upon data for the particular gel polymer being available.

From the hydrodynamic permeability there can be derived the  
30 hydrodynamic radius, defined as the ratio of the pore volume to the pore wetted surface area. It can be calculated from the hydrodynamic (Darcy) permeability using



the Carman-Kozeny equation as given, for example, in the book J. Happel and H. Brenner, Low Reynolds Numbers Hydrodynamics, Noordhof of Int. Publ., Leyden, 1973, p. 393, incorporated by reference herein. It is necessary to assume  
5 a value for the Kozeny constant and for the purpose of these calculations the inventors assume a value of 5. Composite materials of the invention, containing macroporous gels, are found to have a hydrodynamic radius more than three times as high as the hydrodynamic radius of the corresponding  
10 reference material.

From the definition of the hydrodynamic permeability it can be derived that two composite materials of the same thickness will have hydrodynamic fluxes at the same pressure that will have the same ratio as their permeability ratio.

15 The size of macropores in the gel can be within a broad range, from a few nanometers to several hundred nanometers. Preferably, the porous gel constituent of the composite material has macropores of average size between about 10 and about 3000 nm, has volume porosity between 30 and 80 % and a  
20 thickness equal to that of the porous support member. In some embodiments, the average size of the macropores is preferably between 25 and 1500 nm, more preferably between 50 and 1000 nm, and most preferably the average size of the macropores is about 700 nm.

25 In the absence of a support member, the macroporous gels used in the present invention may be non-self supporting, and they may change or even lose their porosity when dried. By inserting the macroporous gel within a porous support member, mechanical strength is conferred upon the  
30 macroporous gel. The utilization of macroporous gels creates a composite material that permits larger molecules,

such as biological molecules, to enter the macropores and the solution containing such molecules to traverse the gel at a high flux.

By a "responsive composite material" is meant a composite material which comprises a macroporous gel whose pore-size can be controlled by varying specific environmental conditions.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an environmental scanning electron microscope (ESEM) image of a macroporous poly(APTAC) gel;

Figure 2 is an ESEM image of a macroporous poly(APTAC) gel incorporated into a support member in the form of a membrane;

Figure 3 is a lysozyme adsorption curve of the membrane prepared in Example 3, below. The membrane volume is 0.467ml;

Figure 4 is a BSA adsorption curve of the membrane prepared in Example 8, below;

Figure 5 is a graphical representation of the hydraulic radius as a function of mass gain with photo- and thermally initiated porous gel containing membranes.  
Gel: poly(glycidyl methacrylate-co-ethylene diacrylate);  
solvents: dodecanol (DDC)/cyclohexanol (CHX) 9/91;

Figure 6 is a graphical representation of the mass gain as a function of total monomer concentration during the preparation of composite membranes;

Figure 7 is an AFM image of the surface of the AM610 membrane; (scanned area: 100  $\mu\text{m}^2$ );

Figure 8 shows ESEM images of a nascent (top) and AM610 (bottom) surfaces; (magnification: 5000x);

Figure 9 shows ESEM images of the surface of AM611 membrane; (magnification: top - 5000x, bottom - 3500x);

- 5 Figure 10 shows ESEM images of membranes AM610 (top) and AM611 (bottom); (magnification 5000x);

Figure 11 is a lysozyme adsorption curve of the membrane prepared in Example 15. The membrane volume is 0.501ml;

- 10 Figure 12 is a lysozyme adsorption curve of the membrane prepared in Example 16. The membrane volume is 0.470ml;

Figure 13 is a lysozyme adsorption curve of the membrane prepared in Example 17. The membrane volume is 0.470ml;

Figure 14 is an ESEM image of a wet macroporous gel that is the product of Example 25;

- 15 Figure 15 is an ESEM image of a wet microporous gel in a fibrous non-woven support member that is the product of Example 26; and

- Figure 16 shows graphically results of using a multi-membrane stack of composite material of Example 22 in a  
20 protein (BSA) adsorption test.

Figure 17 graphically displays the effect of monomer concentration on the mass gain of composite materials.

- Figure 18 graphically displays the effect of ionic interactions on the flux through a composite material at a  
25 pressure of 100 kPa.

Figure 19 graphically displays changes in trans-membrane pressure and permeate conductivity as a function of salt

concentration in the permeate (A and B) and the changes of trans-membrane pressure as a function of permeate conductivity (salt concentration) (C).

Figure 20 shows the relationship between trans-membrane pressure, conductivity and absorbance for the HIGG ultrafiltration carried out in Example 39.

Figure 21 shows the relationship between conductivity and absorbance for the HSA ultrafiltration carried out in Example 39.

Figure 22 shows the relationship between trans-membrane pressure, conductivity and absorbance for the HSA/HIGG ultrafiltration carried out in Example 39.

#### DETAILED DESCRIPTION OF THE INVENTION

##### *General Characteristics*

Preferably, the macroporous gel is anchored within the support member. The term "anchored" is intended to mean that the gel is held within the pores of the support member, but the term is not necessarily restricted to mean that the gel is chemically bound to the pores of the support member. The gel can be held by the physical constraint imposed upon it by enmeshing and intertwining with structural elements of the host, without actually being chemically grafted to the host or support member, although in some embodiments, the macroporous gel may become grafted to the surface of the pores of the support member.

It will be appreciated that as the macropores are present in the gel that fills the pores of the support member, the macropores must be smaller than the pores of the support member. Consequently, the flow characteristics and

separation characteristics of the composite material are dependent on the characteristics of the macroporous gel, but are largely independent of the characteristics of the porous support member, with the proviso, of course, that the size  
5 of the pores present in the support member is greater than the size of the macropores of the gel. The porosity of the composite material can be tailored by filling the support member with a gel whose porosity is primarily controlled by the nature and amounts of monomer or polymer, cross-linking  
10 agent, reaction solvent, and porogen, if used. As pores of the support member are filled with the same macroporous gel material, there is achieved a high degree of consistency in properties of the composite material, and for a particular support member these properties are determined largely, if  
15 not entirely, by the properties of the macroporous gel. The net result is that the invention provides control over macropore-size, permeability and surface area of the composite materials.

The number of macropores in the composite material is not  
20 dictated by the number of pores in the support material. The number of macropores in the composite material can be much greater than the number of pores in the support member, although the macropores are smaller than the pores in the support member. As mentioned above, the effect of the pore-  
25 size of the support material on the pore-size of the macroporous gel is generally quite negligible. An exception to this is found in those cases where the support member has a large difference in pore-size and pore-size distribution, and where a macroporous gel having very small pore-sizes and  
30 a narrow range in pore-size distribution is sought. In these cases, large variations in the pore-size distribution of the support member are weakly reflected in the pore-size distribution of the macroporous gel. As such it is

preferable to use a support member with a somewhat narrow pore-size range in these situations.

The properties of the composite materials can be tuned, by adjusting the average pore diameter of the macroporous gel.

5 For some purposes, for example ultrafiltration by means of size exclusion, small pores may be required. For other purposes, for example use as a solid support for a chemical synthesis involving fast-kinetics, large pores may be required. The size of the macropores is mainly dependent on  
10 the nature and concentration of the cross-linking agent, the nature or the solvent or solvents in which the gel is formed, the amount of any polymerization initiator or catalyst and, if present, the nature and concentration of porogen.

15 Generally, as the concentration of cross-linking agent is increased, the size of the macropores in the gel is also increased. For example, the molar ratio of polyfunctional compound(s) (cross-linking agent) to monomer(s) may be in the range of from about 5:95 to about 70:30, preferably in the  
20 range of from about 10:90 to about 50:50, and more preferably in the range of from about 15:85 to about 45:55.

The components of the macroporous gel are introduced into the pores of the support member by means of a liquid vehicle, and solvent selection for *in situ* polymerization or  
25 cross-linking plays a role in obtaining porous gels. Generally, the solvent or solvent mixture should dissolve monomers and polyfunctional compounds, or cross-linkable polymers and cross-linking agents, over a wide range of concentrations. If the solvent is a good solvent for the  
30 gel polymer, porosity can only be introduced into the gel by cross-linking or porogen. If, however, there is present a

solvent that is a thermodynamically poor solvent or non-solvent, this solvent will act as a porogen. By combining solvents of different affinities to the gel polymer, from a good solvent through a poor solvent to a non-solvent, at different ratios, both porosity and pore dimensions can be altered. In general, the poorer the solvent or the solvent mixture the higher the porosity and the sizes of macropores. Preferably, the solvent or solvent mixture for *in situ* polymerization contains poor solvent in the range from about 0% to about 100%, more preferably from about 10% to about 90%. Examples of good solvents for poly(2-acrylamido-2-methyl-1-propanesulfonic acid) are water and N,N-dimethylformamide. Examples of poor solvents include dioxane, hydrocarbons, esters, and ketones. An example of a good solvent for poly(acrylamide) is water. Examples of poor solvents include dioxane, alcohols such as methanol, N,N-dimethylformamide, hydrocarbons, esters, and ketones. Preferably, the solvents used are miscible with water.

When the polymerization is carried out using a liquid vehicle that contains non-solvents or poor solvents, the resulting structure is often built of clusters of agglomerated microspheres that form the body of the macroporous gel. The pores in such materials consist of the voids located between clusters (macropores), voids between microspheres in the clusters (mesopores), and pores inside the microspheres themselves (micropores).

Porogens can be broadly described as pore generating additives. Examples of porogens that can be used in the gel-forming reaction include thermodynamically poor solvents or extractable polymers, for example poly(ethyleneglycol), or surfactants, or salts. Porogens are known in the art, and a person skilled can determine, using standard

experimental techniques and without exercise of any inventive faculty, which porogens are suitable to prepare macroporous gels for use in a desired composite material.

There is no simple way to predict accurately the structure parameters of porous gels obtained under given conditions, but qualitative rules are available to give some guidance. Generally, the mechanism of porous gel formation via polymerization of one or more monomers and cross-linkers involves, as a first step, an agglomeration of polymer chains to give nuclei. The polymerization continues both in the nuclei and in the remaining solution to form microspheres which grow in size by capturing newly precipitated nuclei and polymers from the solution. At some point, the microspheres become interconnected with each other in large clusters that form the body of the macroporous gel. The poorer the solvent quality the faster nucleation occurs during the gel-forming process. If the number of nuclei formed is very large, as in the case of high concentration of a polymerization initiator, smaller pores may be expected. If, however, the number of nuclei is smaller and the reaction kinetics is such that the nuclei can grow larger, large pores are formed in the gel. High concentration of a cross-linker usually causes early nucleation. The nuclei, however, may be too highly cross-linked to be able to swell with the monomers, grow and coalesce in clusters. This may result in very small pores. Because of the different ways that the polymerization may proceed and the polymerization conditions may affect the gel porous structure, a large variety of structures can be obtained but conditions for each of the structures need to be determined experimentally.



*Separations with the Composite Material*

In some embodiments of the invention the composite material is used as a separating medium, for example in filtration operations where a liquid to be filtered is passed through the composite membrane and separation of one or more components from the liquid is effected by size exclusion in an uncharged macroporous gel. The separation can further be enhanced by the Donnan exclusion of charged molecules by use of a charged macroporous gel. If the macroporous gel contains a fixed charge and the charge of the solutes can be appropriately adjusted, the solutes can be separated even against their size gradient. For example with a solution containing a mixture of proteins, if a pH value is selected for which one of the proteins in the mixture is at its isoelectric point while the other proteins retain charge of the same sign as the membrane charge, the other proteins can be held back in the retentate because of the charge repulsion with the membrane. By tailoring the conditions for fractionation, good selectivity, even for proteins of the same size, can be obtained.

Separation can also be achieved by the presence of reactive functional groups in the macroporous gel. These functional groups can be used to bear a ligand or other specific binding site that has an affinity to a molecule or ion, including a biomolecule or biomolecular ion. When a liquid containing the particular molecule or ion is passed through the composite material the ligand or specific binding site interacts with the molecule or ion enough to adsorb it. In some cases it is possible to subsequently desorb the captured molecule or ion when the environment around the composite material is subsequently altered, for example by changing the nature of the solvent passed through the

macropores of the gel. The binding sites can also include charged groups.

### *Composition of the Macroporous Gels*

The macroporous gels can be formed through the in-situ  
5 reaction of one or more polymerisable monomers with one or  
more cross-linkers, or of one or more cross-linkable  
polymers with one or more cross-linker to form a cross-  
linked gel that has macropores of a suitable size. Suitable  
polymerisable monomers include monomers containing vinyl or  
10 acryl groups. For Donnan exclusion, there can be used vinyl  
or acryl monomers containing at least one polar and/or ionic  
functional group, or functional group that can be converted  
into ionic group. For biological affinity there can be used  
vinyl or acryl monomers containing at least one reactive  
15 functional group. Preferred polymerisable monomers include  
acrylamide, 2-acryloxyethyltrimethylammonium chloride,  
N-acryloxysuccinimide,  
N-acryloyltris(hydroxymethyl)methylamine, 2-aminoethyl  
methacrylate hydrochloride, N-(3-aminopropyl)methacrylamide  
20 hydrochloride, butyl acrylate and methacrylate,  
N,N-diethylacrylamide, N,N-dimethylacrylamide,  
2-(N,N-dimethylamino)ethyl acrylate and methacrylate,  
N-[3-(N,N-dimethylamino)propyl]methacrylamide,  
N,N-dimethylacrylamide, n-dodecyl acrylate, n-dodecyl  
25 methacrylate, dodecyl methacrylamide, ethyl methacrylate,  
2-(2-ethoxyethoxy)ethyl acrylate and methacrylate,  
2,3-dihydroxypropyl acrylate and methacrylate, glycidyl  
acrylate and methacrylate, n-heptyl acrylate and  
methacrylate, 1-hexadecyl acrylate and methacrylate,  
30 2-hydroxyethyl acrylate and methacrylate,  
N-(2-hydroxypropyl)methacrylamide, hydroxypropyl acrylate  
and methacrylate, methacrylamide, methacrylic anhydride,

methacryloxyethyltrimethylammonium chloride,  
2-(2-methoxy)ethyl acrylate and methacrylate, octadecyl  
acrylamide, octylacrylamide, octyl methacrylate, propyl  
acrylate and methacrylate, N-iso-propylacrylamide, stearyl  
5 acrylate, styrene, 4-vinylpyridine, vinylsulfonic acid,  
N-vinyl-2-pyrrolidinone. Particularly preferred monomers  
include dimethyldiallylammonium chloride, acrylamido-2-  
methyl-1-propanesulfonic acid (AMPS), (3-acrylamidopropyl)  
trimethylammonium chloride (APTAC), acrylamide, methacrylic  
10 acid (MAA), acrylic acid (AA), 4-styrenesulfonic acid and  
its salts, acrylamide, glycidyl methacrylate,  
diallylamine, and diallylammonium chloride.

The crosslinker may be, for example, a compound containing  
at least two vinyl or acryl groups. Examples of crosslinkers  
15 include bisacrylamidoacetic acid, 2,2-bis[4-(2-  
acryloxyethoxy)phenyl]propane, 2,2-bis(4-  
methacryloxyphenyl)propane, butanediol diacrylate and  
dimethacrylate, 1,4-butanediol divinyl ether,  
1,4-cyclohexanediol diacrylate and dimethacrylate,  
20 1,10-dodecanediol diacrylate and dimethacrylate,  
1,4-diacryloylpiperazine, diallylphthalate,  
2,2-dimethylpropanediol diacrylate and dimethacrylate,  
dipentaerythritol pentaacrylate, dipropylene glycol  
diacrylate and dimethacrylate,  
25 N,N-dodecamethylenebisacrylamide, divinylbenzene, glycerol  
trimethacrylate, glycerol tris(acryloxypropyl) ether,  
N,N'-hexamethylenebisacrylamide,  
N,N'-octamethylenebisacrylamide, 1,5-pentanediol diacrylate  
and dimethacrylate, 1,3-phenylenediacylate, poly(ethylene  
30 glycol) diacrylate and dimethacrylate, poly(propylene)  
diacylate and dimethacrylate, triethylene glycol diacylate  
and dimethacrylate, triethylene glycol divinyl ether,  
tripropylene glycol diacylate or dimethacrylate, diallyl

diglycol carbonate, poly(ethylene glycol) divinyl ether, N,N'-dimethacryloylpiperazine, divinyl glycol, ethylene glycol diacrylate, ethylene glycol dimethacrylate, N,N'-methylenebisacrylamide, 1,1,1-trimethylolethane

5 trimethacrylate, 1,1,1-trimethylolpropane triacrylate, 1,1,1-trimethylolpropane trimethacrylate, vinyl acrylate, 1,6-hexanediol diacrylate and dimethacrylate, 1,3-butylene glycol diacrylate and dimethacrylate, alkoxyated cyclohexane dimethanol dicarylate, alkoxyated hexanediol

10 diacrylate, alkoxyated neopentyl glycol diacrylate, aromatic dimethacrylate, caprolacone modified neopentylglycol hydroxypivalate diacrylate, cyclohexane dimethanol diacrylate and dimethacrylate, ethoxyated bisphenol diacrylate and dimethacrylate, neopentyl glycol

15 diacrylate and dimethacrylate, ethoxyated trimethylolpropane triarylate, propoxyated trimethylolpropane triacrylate, propoxyated glyceryl triacrylate, pentaerythritol triacrylate, tris (2-hydroxy ethyl)isocyanurate triacrylate, di-trimethylolpropane

20 tetraacrylate, dipentaerythritol pentaacrylate, ethoxyated pentaerythritol tetraacrylate, pentaacrylate ester, pentaerythritol tetraacrylate, and caprolactone modified dipentaerythritol hexaacrylate. Particularly preferred cross-linking agents include N,N',-methylenebisacrylamide,

25 diethylene glycol diacrylate and dimethacrylate, trimethylolpropane triacrylate, ethylene glycol diacrylate and dimethacrylate, tetra(ethylene glycol) diacrylate, 1,6-hexanediol diacrylate, divinylbenzene, poly(ethylene glycol) diacrylate.

30 The concentration of monomer in the macroporous gel can have an effect on the resiliency of the macroporous gel prepared. A low monomer concentration can lead to a macroporous gel that is non-self supporting. Such non-self supporting gels

might be advantageous as adsorbents, as they could lead to gels having greater adsorption capacity. In some embodiments, the monomer concentration is 60% or less, for example about 60, 50, 40, 30, 20, 10 or 5%.

5 When a cross-linkable polymer is used, it can be dissolved and reacted in-situ in the support with a cross-linking agent to form the macroporous gel. Suitable cross-linkable polymers include poly(ethyleneimine), poly(4-vinylpyridine), poly(vinylbenzyl chloride), poly(diallylammonium chloride),  
10 poly(glycidyl methacrylate), poly(allylamine), copolymers of vinylpyridine and dimethyldiallylammonium chloride, copolymers of vinylpyridine, dimethyladiallylammonium chloride, or (3-acrylamidopropyl)trimethylammonium chloride with glycidyl acrylate or methacrylate, of which  
15 poly(ethyleneimine), poly(diallylammonium chloride), and poly(glycidyl methacrylate) are preferred. Use of cross-linkable polymers instead of monomers can, in some instances, require a decrease in the concentration of cross-linking agent. In order to retain the large size of the  
20 pores in the gel with a lower cross-linking agent concentration, a porogen can be added to the mixture used to prepare the macroporous gel.

The cross-linking agent for reaction with the cross-linkable polymer is selected from molecules containing two or more  
25 reactive groups that can react with an atom or group of atoms in the polymer to be cross-linked, such as epoxy groups or alkyl/aryl halides that can react with nitrogen atoms of polyamines, or amine groups that can react with alkyl/aryl halides or epoxy groups of glycidyl-group-  
30 containing polymers to be in situ cross-linked. Suitable cross-linkers include ethylene glycol diglycidyl ether, poly(propylene glycol) diglycidyl ether, 1,3-dibromopropane,

1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibromohexane,  
 $\alpha,\alpha'$ -dibromo-p-xylene,  $\alpha,\alpha'$ -dichloro-p-xylene, 1,4-dibromo-  
2-butene, piperazine, 1,4-diazabicyclo[2.2.2]octane,  
1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane,  
5 1,5-diaminopentane, 1,6-diaminohexane, 1,7-diaminoheptane,  
1,8-diaminooctane.

It is also possible to modify polymers containing reactive  
groups such as an amino, hydroxyl, carboxylic acid,  
carboxylic acid ester, or epoxy groups with reagents to  
10 introduce vinyl groups that can be subsequently polymerized  
by treatment with a polymerization initiator to form a  
macroporous gel. Examples of suitable vinyl groups that can  
be introduced include vinylbenzene derivatives, allyl  
derivatives, acrolyl and methacrolyl derivatives. The  
15 cross-linking of these vinyl substituted polymers can in  
some instances be facilitated by the introduction of further  
monomers such as acrylamide, N-vinylpyrrolidone, acrylic and  
methacrylic acids and their salts.

Macromonomers can also be used as monomers or as cross-  
20 linking agents. Macromonomers can be polymers or oligomers  
that have one (monofunctional) or more (cross-linking agent)  
reactive groups, often at the ends, which enable them to act  
as a monomer or a cross-linker. For monomers, each  
macromonomer molecule is attached to the main chain of the  
25 final polymer by reaction of only one monomeric unit in the  
macromonomer molecule. Examples of macromonomers include  
poly(ethylene glycol)acrylate and poly(ethylene  
glycol)methacrylate, while examples of polyfunctional  
macromonomers include poly(ethylene glycol)diacrylate and  
30 poly(ethylene glycol)dimethacrylate. Macromonomers  
preferably have molecular weights of about 200 Da or  
greater.

Many macroporous gels can be prepared, including neutral hydrogels, charged hydrogels, polyelectrolyte gels, hydrophobic gels, and neutral and functional gels.

If the gel selected is a neutral hydrogel or a charged hydrogel for which water is the swelling liquid medium, the resulting supported macroporous gel is normally quite hydrophilic. Hydrophilic composite materials are preferred as they provide better flow characteristics and impart anti-fouling tendencies to the membranes. Examples of suitable hydrogels include cross-linked gels of poly(vinyl alcohol), poly(acrylamide), poly(isopropylacrylamide), poly(vinylpyrrolidone), poly(hydroxymethyl acrylate), poly(ethylene oxide), copolymers of acrylic acid or methacrylic acid with acrylamide, isopropylacrylamide, or vinylpyrrolidone, copolymers of acrylamide-2-methyl-1-propanesulfonic acid with acrylamide, isopropylacrylamide, or vinylpyrrolidone, copolymers of (3-acrylamidopropyl) trimethylammonium chloride with acrylamide, isopropylacrylamide, or N-vinylpyrrolidone, copolymers of diallyldimethylammonium chloride with acrylamide, isopropylacrylamide, or vinylpyrrolidone. Preferred hydrogels include cross-linked poly(vinyl alcohol), poly(acrylamide), poly(isopropylacrylamide) and poly(vinylpyrrolidone) and cross-linked copolymers of neutral monomers such as acrylamide or N-vinylpyrrolidone with charged monomers such as acrylamide-2-methyl-1-propanesulfonic acid or diallyldimethylammonium chloride.

The macroporous gels can be selected to comprise polyelectrolytes. Like the charged hydrogels, polyelectrolyte gels give hydrophilic composite material, and they also carry a charge. The polyelectrolyte gel can be selected, for example, from cross-linked poly(acrylamido-

2-methyl-1-propanesulfonic acid) and its salts, poly(acrylic acid) and its salts, poly(methacrylic acid) and its salts, poly(styrenesulfonic acid) and its salts, poly(vinylsulfonic acid) and its salts, poly(alginic acid) and its salts, 5 poly[(3-acrylamidopropyl)trimethylammonium] salts, poly(diallyldimethylammonium) salts, poly(4-vinyl-N-methylpyridinium) salts, poly(vinylbenzyl-N-trimethylammonium) salts, poly(ethyleneimine) and its salts. Preferred charged gels include cross-linked poly(acrylic 10 acid) and its salts, poly(methacrylic acid) and its salts, poly(acrylamido-2-methyl-1-propanesulfonic acid) and its salts, poly[(3-acrylamidopropyl)trimethylammonium] salts, poly(diallyldimethylammonium) salts, and poly(4-vinylpyridinium) salts.

15 One of the differences between charged gels and polyelectrolyte gels is that the repeating monomer in the polyelectrolyte gel bears a charge, while in the charged gel, the charge is found in a co-polymerized unit that is randomly distributed through the polymer. The monomer used 20 to form the polyelectrolyte gel or the co-polymer in the charged gel that bears a charge usually has a charge bearing group, but it can also be a non-charge-bearing group that can become charged in a post-gelation process (e.g. quaternization of nitrogen bearing groups). Examples of 25 polymers that can become charged include poly(4-vinylpyridine) which can be quaternized with various alkyl and alkylaryl halides. Suitable alkyl halides include those having up to 8 carbon atoms, for example methyl iodide, ethyl bromide, butyl bromide, and propyl bromide. Suitable 30 alkylaryl halides include benzyl halides, especially benzyl chloride and benzyl bromide. Another polymer that can become charged is poly(vinylbenzyl chloride), which can be quaternized with various amines, for example, lower



alkylamines or aromatic amines such as triethylamine, pyridine, azabicyclo[2.2.2]octane, N-methylpyrrolidine, and N-methylpiperidine, and lower hydroxyalkylamines, for example triethanolamine. Yet another polymer that can  
5 become charged is poly(glycidyl methacrylate) or poly(glycidyl acrylate), which can react with various amines, for example lower alkylamines such as diethylamine and triethylamine, azabicyclo[2.2.2]octane, N-methylpyrrolidine, and N-methylpiperidine. Alternatively,  
10 glycidyl moieties can be converted to sulfonic acid groups by reaction with, for example alkali metal sulfites such as sodium sulfite. A person skilled in the art will appreciate that there are other polymers that are, or can be rendered, charge-bearing.

15 The macroporous gel can be selected to comprise hydrophobic monomers to permit separations in organic solvents, for example hydrocarbons, especially liquid paraffins such as hexanes. Hydrophobic monomers, such as styrene and its derivatives, for example an alkyl substituted styrene  
20 derivative such as para-tertbutyl styrene, can be used to prepare hydrophobic macroporous gels. Copolymers of these monomers can be used.

A macroporous gel comprising hydrophobic monomers can be used to capture molecules from fluids passing through the  
25 pores by hydrophobic interactions.

As stated above, the macroporous gels can also be selected to comprise reactive functional groups that can be used to attach ligands or other specific binding sites. These functional macroporous gels can be prepared from cross-  
30 linked polymers bearing functional groups, for example epoxy, anhydride, azide, reactive halogen, or acid chloride

groups, that can be used to attach the ligands or other specific binding sites. Examples include cross-linked poly(glycidyl methacrylate), poly(acrylamidoxime), poly(acrylic anhydride), poly(azelaic anhydride),  
5 poly(maleic anhydride), poly(hydrazide), poly(acryloyl chloride), poly(2-bromoethyl methacrylate), poly(vinyl methyl ketone). Functionality that can be introduced can take the form of antibodies or fragments of antibodies, or alternatively, chemical mimics such as dyes. Functional  
10 gels are attractive in biomolecule purifications or separations, as they can offer preferential binding to certain molecules by binding to active sites, while being non-reactive to other molecules, even when there is no significant difference in size between the molecules,  
15 examples being affinity ligands selected to bind with some proteins but not others. Affinity ligands that can be attached to porous gels via reactive groups include amino acid ligands such as L-phenylalanine, tryptophan, or L-histidine to separate  $\gamma$ -globulins and immunoglobulins,  
20 antigen and antibody ligands such as monoclonal antibodies, protein A, recombinant protein A, protein G, or recombinant protein G to separate immunoglobulins from different media, dye ligands such as cibaron blue or active red to separate albumins and various enzymes, metal affinity ligands such as  
25 complexes of iminodiacetic acid (IDA) ligand with  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , or  $\text{Co}^{2+}$  to separate various proteins such as histidine, lysozyme, or tryptophan from various media.

#### *Responsive Macroporous Gels*

Polymers that change their conformation in response to  
30 changes in environmental conditions are known. By incorporating the properties of such polymers in the macroporous gel, a composite material with dynamic pore-size

is obtained. These composite materials having responsive characteristics are substantially the same as the composite materials described above, except that at least one of the monomers or polymers that form the macroporous gel has a chemical structure which facilitates changes in pore-size.

The changes in the pore-size of the macroporous gel are due to the physical relationship between the support member and the macroporous gel. The composite material can be described as having three distinct zones: (a) the support member, which ideally does not change shape, (b) the incorporated macroporous gel that "fills" the pores of the support member, and (c) the volume within the macropores of the gel, which volume is filled with water or a solvent and in which is found very little or no gel polymer. Under pressure, hydraulic flow occurs through the macropores of the gel, and the flux through the composite material is related to the number of pores in the macroporous gel, the radius of these pores, and the tortuosity of the path of the pores in the macroporous gel through the composite material.

As the degree of swelling of the macroporous gel is changed by an environmental stimulus, the total volume occupied by the macroporous gel is constrained by the fixed total volume defined by the support member. As the overall volume of the macroporous gel is constrained by the support member, by necessity the volume fraction of the gel expands into the area defined by macropores in the gel. As the number of macropores and their tortuosity remain essentially constant with the change in volume fraction of the macroporous gel, the diameter or radius of the macropores themselves must change. If the macroporous or structured gel were unconfined, the environmentally induced changes would cause the total volume of the swollen gel to change. As such it

would not follow in this unconfined case that the changes would result in a controllable change in pore-size of the macroporous gel.

The reason behind the change in volume of the macroporous gel is related to interactions between the polymer structures that form the gels, or the interactions between the polymer chains and the solvents or solutes present in the solvent that diffuse into the gel. The changes in the volume occupied by the gel are linked to the conformation adopted by the polymer chains that form the macroporous gels. The natural tendency of the polymer chains is to coil around themselves, which leads to a gel having a smaller volume. If the polymer chains within the gel can be manipulated to uncoil and form a more rigid backbone, the overall volume of the gel will increase. It is thus this coiling/uncoiling which is affected by the environmental stimuli that are applied to the responsive composite material.

The volume changes of the pores can either be "continuous" or "discontinuous". A continuous volume change takes place over a relatively large change in the triggering environmental condition and where there exists at least one stable volume near the transition between the swollen and collapsed state. Preferably, a continuous volume change will go through a multitude of stable transition volumes between the swollen and the collapsed state. A discontinuous volume change in gels is characterised by the reversible transition from swollen to collapsed state taking place over an extremely small change in the triggering environmental condition, for example, less than 0.1 pH unit or 0.1 degree Celsius. Gels exhibiting discontinuous volume change are called "phase-transition" gels and systems or

devices with such gels are often called "chemical valves". Preferably, the responsive macroporous gels according to this embodiment of the invention undergo a "continuous" volume change through discrete stable volumes that can be  
5 utilized to control the pore-size of the gel.

Of the environmental stimuli that can be used to change the pore-size in the responsive macroporous, mention is made of pH, specific ions, ionic strength, temperature, light, electric fields, and magnetic fields. The effect of each  
10 stimulus, and examples of monomers that react to such a stimulus, will be described in more detail below.

One stimulus that can be utilised to change the pore-size of responsive macroporous gel is the pH of the solution being passed through the pores of the gel. A change in the pH of  
15 the solution will affect the pore-size of the gel if the gel comprises weak acids or weak bases. In such cases, the natural tendency of the polymer chain within the gel to coil around itself will be balanced by the repulsion between the charged groups (weak acidic or basic groups) along the  
20 length of the polymer chain. Variations in the amount of charge along the chain cause large changes in conformation of the polymer chain, which in turn causes changes in the volume occupied by the gel. Changes in the pH of the solution are effective at controlling the amount of  
25 repulsion along the polymer chain, as they change the degree of ionisation of the charged groups. A gel comprising weak acid groups becomes less ionised as the pH is lowered and the gel contracts. Conversely, a weak base becomes more ionised as the pH is lowered and the chain elongates or  
30 stretches to give a swollen gel.

Examples of monomers that have weak acid functionality include acrylic acid, methacrylic acid, itaconic acid, 4-vinylbenzoic acid, bisacrylamidoacetic acid, and bis(2-methacryloxyethyl) phosphate. Examples of monomers that have weak base functionality include 2-aminoethyl methacrylate hydrochloride, N-(3-aminopropyl)methacrylamide hydrochloride, 2-(tert-butylamino)ethyl methacrylate, diallylamine, 2-(N,N-diethylamino)ethyl methacrylate, 2-(N,N-dimethylamino)ethyl acrylate, 2-(N,N-dimethylamino)ethyl methacrylate, 1-vinylimidazole, and 4-vinylpyridine. Glycidyl methacrylate derivatized hyaluronate-hydroxyethyl acrylate based hydrogels can also be used to prepare composite materials that are pH responsive [Inukai M., Jin Y., Yomota C., Yonese M., *Chem. Pharm. Bull.*, (2000), 48:850-854; which is hereby incorporated by reference].

Variations in pH have little effect on the degree of ionisation of strong acids and bases, and as such, only drastic variations in pH can effect pore-size changes in gels comprising these functionalities.

Another stimulus that can be utilised for changing the pore-size of a responsive macroporous gel is the salt concentration of the solution being passed through the pores of the gel. Similarly to variations in pH, variations in salt concentration will effect pore-size variations in macroporous gels that comprise weak acidic or weak basic groups. The reason for the changes in pore-size, however, does differ slightly. The addition of an ionic solute has the ability to shield the charged groups found on the polymer chain in the gel by the formation of ion-pairs. This lessens the coulombic repulsion between the adjacent charged groups, which allows the chain to relax into a

coiled conformation. An increase in salt concentration will shield both a weak acid group and a weak base group. Therefore, when the salt concentration is increased, for example by adding a concentrated salt solution to the bulk solution being passed through the composite material, the shielding effect of the additional ions leads to an increase in pore size. Alternatively, a decrease in salt concentration, such as obtained by diluting the bulk solution being passed through the composite material, will lead to less shielding and a smaller pore size.

Changes in salt concentration can also be used with macroporous gels that comprise strong acid groups and strong basic groups, as these groups are also shielded by the presence of free ionic species.

Examples of monomers that bear weak acid or base groups are listed above. Examples of monomers that have strong acid functionality include 2-acrylamido-2-methyl-1-propanesulfonic acid, sodium 2-methyl-2-propene-1-sulfonate, sodium styrenesulfonate, and sodium vinylsulfonate.

Examples of monomers that have strong basic functionality include 2-acryloxyethyltrimethylammonium chloride, diallyldimethylammonium chloride, methacrylamidopropyltrimethylammonium chloride, and 3-methacryloxy-2-hydroxypropyltrimethylammonium chloride.

Ionic functionality (weak/strong acids and bases) may also be introduced in macroporous gels that do not originally bear charged functionalities but that bear instead reactive groups that can be converted into ionic or ionisable moieties in a post-polymerization treatment. Suitable monomers with reactive groups include acrylic anhydride, allyl glycidyl ether, bromostyrene, chloromethylstyrene,

chlorostyrenes, glycidyl acrylate, glycidyl methacrylate, 4-hydroxybutyl methacrylate, 2-hydroxyethyl acrylate, methacryloyl chloride. For example, a macroporous gel comprising a glycidyl acrylate or methacrylate group can be  
5 treated with diethylamine to introduce weak base functionality or with sodium sulfite in an iso-propanol/water mixture to introduce strong acid (sulfonic acid) functionality.

Another stimulus that can be used to change the pore-size of  
10 a responsive macroporous gel is the temperature of the gel. Various methods are available for changing the temperature of the macroporous gel, one of which includes changing the temperature of a liquid flowing through the pores of the macroporous gel. While the change in overall gel volume for  
15 temperature dependant gels is again due to the control of the coiling or uncoiling of the polymer chains that form the gel, the contraction or expansion of the gel is not linked to the presence of charged groups on the polymer chain. For temperature dependant gels, the amount solvation of the  
20 polymer chain controls the conformation of the polymer chain. At lower temperatures the chains are solvated, which allows an elongated conformation of the polymer chain. As the temperature is increased, an entropic desolvation takes place causing the chains to coil and contract. Therefore,  
25 increases in temperature lead to larger pore sizes in the gel while decreases in temperature lead to smaller pore sizes.

Macroporous gels that comprise hydrophobic monomers are most suitable for use in temperature dependant systems, as  
30 solvation effects are markedly observed for polymers that have hydrophobic functionality. Examples of monomers that have hydrophobic functionality include N-(propyl)acrylamide,



N-(tert-butyl)acrylamide, butyl acrylates, decyl acrylates, decyl methacrylates, 2-ethylbutyl methacrylate, n-heptyl acrylate, n-heptyl methacrylate, 1-hexadecyl acrylate, 1-hexadecyl methacrylate, n-hexyl acrylate, n-hexyl methacrylate, and N-(n-octadecyl)acrylamide. Gels displaying thermal response can also be prepared from sulphated hyaluronic acid-based gels (see Barbucci R., Rappuoli R., Borzacchiello A., Ambrosio L., *J. Biomater. Sci.-Polym. Ed.*, (2000), 11:383-399), incorporated herein by reference.

Light is another stimulus that can be used to change the pore-size of the responsive macroporous gel. Light induced changes are due to photoisomerizations in the backbone or the side-chains of the polymer chains that form the gel. These photoisomerizations cause a change in either the conformation and local dipole moment, or in the degree of ionisation through light induced electron transfer reactions. One type of monomer that is suitable for use in light controlled systems comprises unsaturated functionalities than can undergo a trans-cis isomerization on irradiation. Examples of photoresponsive monomers that go through cis-trans conformation and dipole changes include 4-(4-oxy-4'-cyanoazobenzene)but-1-yl methacrylate, 6-(4-oxy-4'-cyanoazobenzene)hex-1-yl methacrylate, 8-(4-oxy-4'-cyanoazobenzene)oct-1-yl methacrylate, 4-[ $\omega$ -methacryloyloxyoligo(ethyleneglycol)]-4'-cyanoazobenzene, 4-methacryloyloxy-4'-{2-cyano-3-oxy-3-[ $\omega$ -methoxyoligo(ethyleneglycol)]prop-1-en-1-yl}azobenzene, and methacrylate monomers containing a mesogenic group and a photochromic para-nitroazobenzene group. It is also possible to incorporate the photoresponsive moiety in the crosslinker instead of the monomer. Examples of

photoresponsive crosslinkers that go through cis-trans conformation and dipole changes include

4,4'-Divinylazobenzene, N, N'-bis( $\beta$ -styrylsulfonyl)-4,4'-diaminoazobenzene, 4,4'-bis(methacryloylamino)azobenzene,  
5 4,4'-dimethacryloylazobenzene, and  
bis((methacryloyloxy)methyl)spirobenzopyran.

The pore-size of the gel can also be altered by subjecting the macroporous gel to an electric field or to an electrical current. The response of the gel to electrochemical current  
10 changes is closely related to the pH systems described above. This close relationship is due to the fact that the passage of an electrochemical current through an aqueous system causes a "water splitting" reaction, which reaction leads to changes in the pH of the aqueous system.

15 Electrical current can be passed through a composite material of the invention e.g. by placing an electrode at either end of the composite material. When current differential is applied to the electrodes, water molecules will separate and concentrations of  $H^+$  and  $HO^-$  will increase  
20 at their respective electrodes. As described earlier, changes in pH can be used to control the pore-size of macroporous gels that comprise weak acid or weak base functionalities, which control is linked to the relationship between the ionisation of these functionalities and the  
25 coiling/uncoiling of the polymer chains that form the gel. Examples of weak acidic and weak basic monomers are given above.

Changes in gel volume due to fluctuations in an electrical field have been previously observed, such as in Murdan S.,  
30 *J. Control. Release*, (2003), 92:1-17; and in Jensen M., Hansen P.B., Murdan S., Frokjaer S., Florence A.T., *Eur. J. Pharm. Sci.*, (2002), 15:139-148, which are hereby

incorporated by reference. While the exact process through which the gel volume is changed by the application of an electrical field is not yet well defined, the volume change itself is well documented. Chondroitin 4-sulphate (CS) is  
5 an example of a monomer that is responsive to electrical field fluctuations.

In some embodiments, the various stimuli response systems can be combined to offer gels that respond to more than one stimulus. An example of such a combined system can be  
10 prepared by combining a charged polymer (weak/strong acid or base) with a hydrophobic monomer. The macroporous gel resulting from such a combination will display responses to changes in salt concentration, changes in solution pH (when weak acids or bases are used), and changes in temperature.  
15 When combining different monomers, it is possible that the responsiveness of the gel to a single of the stimuli will be diminished, as the concentration of the monomer that responds to that particular stimulus will be lowered in the gel.  
20 The magnitude of the response expressed by the macroporous gels, when various stimuli are applied to the gel, depends many different variables, a few of which are discussed below:

The responsiveness of the macroporous gel is dependent on  
25 the concentration of the crosslinking agent. Generally, as the concentration of cross-linking agent is increased, the size of the macropores in the responsive gel is also increased, but the range of pore-size changes is decreased. This relationship is fairly straightforward, as a higher  
30 concentration of crosslinks within the gel will limit the amount of coiling and uncoiling that will be available to

the responsive gel. The molar ratio of crosslinking agent(s) to monomer(s) may be in the range of from about 5:95 to about 40:60, preferably in the range of from about 7.5:92.5 to about 10:90, and more preferably in the range of  
5 from about 10:90 to about 25:75.

Certain stimuli naturally evoke a broader range of response in the gel, as they more effectively affect the conformation of the polymer chains that form the gel. For example, variations in pH or temperature evoke a strong response from  
10 the appropriate macroporous gels, while changes in salt concentrations and light intensity evoke a slightly smaller response.

The concentration of the responsive monomer in the gel also affects the level of response demonstrated by the gel.  
15 Preferably, the responsive macroporous gels are composed of one or more responsive monomers and of one or more neutral monomers. The presence of a neutral monomer is important in those systems that have a very strong response to changes in the environmental conditions, as such systems often display  
20 discontinuous responses in pore-size (valve-effects). Addition of a neutral monomer attenuates the response, permitting a more controlled change in pore-size. Preferably, the molar ratio of the neutral monomers to the molar ratio of responsive monomers in the responsive  
25 macroporous gel is in the range from 5:95 to 95:5, more preferably in the range from 25:75 to 75:25, and more preferably in the range from 40:60 to 60:40. Suitable neutral monomers include acrylamide, N-acryloylmorpholine, N-acryloxysuccinimide, 2-acrylamido-2-(hydroxymethyl)-1,3-propanediol, N,N-diethylacrylamide, N,N-dimethylacrylamide,  
30 2-(2-ethoxyethoxy)ethyl acrylate, 2-ethoxyethyl methacrylate, 2,3-dihydroxypropyl methacrylate,

2-hydroxyethyl methacrylate,  
N-(2-hydroxypropyl)methacrylamide, hydroxypropyl  
methacrylate, methacrylamide, N-[tris(hydroxymethyl)methyl]-  
1-methacrylamide, N-methylmethacrylamide, N-methyl-N-  
5 vinylacetamide, poly(ethylene glycol) monomethacrylate,  
N-iso-propylacrylamide, N-vinyl-2-pyrrolidone.

*Porous Support Member*

A variety of materials can be used to form the support  
member; however, apart from materials such as cellulose and  
10 some of its derivatives, most of these materials are  
strongly or relatively hydrophobic. Hydrophobic filtration  
membranes are not usually desired for use with aqueous  
systems, as they can lead to higher membrane fouling  
tendencies. The more inert and cheaper polymers such as  
15 polyolefins, for example (poly(ethylene), poly(propylene)  
poly(vinylidene difluoride)) can be used to make microporous  
membranes, but these materials are very hydrophobic. In  
some embodiments of the present invention, the  
hydrophobicity of the support member does not affect the  
20 degree of fouling experienced by the composite material as  
the flow of liquid through the composite material takes  
place primarily in the macropores of the gel.

In some embodiments, the porous support member is made of  
polymeric material and contains pores of average size  
25 between about 0.1 and about 25  $\mu\text{m}$ , and a volume porosity  
between 40 and 90 %. Many porous substrates or membranes  
can be used as the support member but the support is  
preferably a polymeric material, and it is more preferably a  
polyolefin, which, while hydrophobic, is available at low  
30 cost. Extended polyolefin membranes made by thermally  
induced phase separation (TIPS), or non-solvent induced

phase separation are mentioned. Hydrophilic supports can also be used, including natural polymers such as cellulose and its derivatives. Examples of suitable supports include SUPOR® polyethersulfone membranes manufactured by Pall Corporation, Cole-Parmer® Teflon® membranes, Cole-Parmer® nylon membranes, cellulose ester membranes manufactured by Gelman Sciences, Whatman® filter and papers.

In some other embodiments the porous support is composed of woven or non-woven fibrous material, for example a polyolefin such as polypropylene. An example of a polypropylene non-woven material is commercially available as TR2611A from Hollingsworth and Vose Company. Such fibrous woven or non-woven support members can have pore sizes larger than the TIPS support members, in some instances up to about 75µm. The larger pores in the support member permit formation of composite materials having larger macropores in the macroporous gel. Composite materials with larger macropores can be used, for example, as supports on which cell growth can be carried out. Non-polymeric support members can also be used, such as ceramic-based supports. The porous support member can take various shapes and sizes.

In some embodiments, the support member is in the form of a membrane that has a thickness of from about 10 to about 2000 µm, more preferably from 10 to 1000µm, and most preferably from 10 to 500µm. In other embodiments, multiple porous support units can be combined, for example, by stacking. In one embodiment, a stack of porous support membranes, for example from 2 to 10 membranes, can be assembled before the macroporous gel is formed within the void of the porous support. In another embodiment, single support member units are used to form composite material membranes, which are then stacked before use.

*Preparation of Composite Materials*

The composite materials of the invention can be prepared by simple, single step methods. These methods can, in some instances, use water or other benign solvents, such as methanol, as the reaction solvent. The methods also have the benefit of using rapid processes that lead to easier and continuous manufacturing possibilities. The composite material is also potentially cheap.

The composite materials of the invention can be prepared, for example, by mixing one or more monomers, one or more polymers, or mixtures thereof, one or more cross-linking agents, optionally one or more initiators and optionally one or more porogens, in one or more suitable solvents. The solution produced is preferably homogeneous, but a slightly heterogeneous solution can be used. The mixture is then introduced into a suitable porous support, where a gel forming reaction takes place. Suitable solvents for the gel forming reaction include, for example, water, dioxane, dimethylsulfoxide (DMSO), dimethylformamide (DMF), acetone, ethanol, N-methylpyrrolidone (NMP), tetrahydrofuran (THF), ethyl acetate, acetonitrile, toluene, xylenes, hexane, N-methylacetamide, propanol, and methanol. It is preferable to use solvents that have a higher boiling point, as these solvents reduce flammability and facilitate manufacture. It is also preferable that the solvents have a low toxicity, and they can be readily disposed of after use. An example of such a solvent is dipropyleneglycol monomethyl ether (DPM).

In some embodiments, it is possible to use dibasic esters (esters of a mixture of dibasic acids) as the solvent.

Dibasic esters (DBEs) are especially suitable for preparing

gels based on polyacrylamide monomers. This solvent system has an unexpected characteristic in that it is poorly soluble in water, which differs from the other solvents used which are essentially completely water miscible. While  
5 water miscible solvents offer advantages in terms of solvent removal after fabrication, water immiscible solvents such as DBE's are good replacements, in certain cases, for solvents such as dioxane that are volatile, flammable, and toxic.

In some embodiments, components of the gel forming reaction  
10 react spontaneously at room temperature to form the macroporous gel. In other embodiments, the gel forming reaction must be initiated. The gel forming reaction can be initiated by any known method, for example through thermal activation or U.V. irradiation. The reaction is more  
15 preferably initiated by U.V. irradiation in the presence of a photoinitiator, as this method has been found to produce larger macropores in the gel, and it accelerates the gel forming reaction more than the thermal activation method. Many suitable photoinitiators can be used, of which  
20 2-hydroxy-1[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959\*), and 2,2-dimethoxy-2-phenylacetophenone (DMPA) are preferred. Other suitable photoinitiators include benzophenone, benzoin and benzoin ethers such as benzoin ethyl ether and benzoin methyl ether,  
25 dialkoxyacetophenones, hydroxyalkylphenones,  $\alpha$ -hydroxymethyl benzoin sulfonic esters. Thermal activation requires the addition of a thermal initiator. Suitable thermal initiators include 1,1'-azobis(cyclohexanecarbonitrile) (VAZO<sup>®</sup> catalyst 88), azobis(isobutyronitrile) (AIBN),  
30 potassium persulfate, ammonium persulfate, and benzoyl peroxide.



If the reaction is to be initiated by U.V. irradiation, a photoinitiator is added to the reactants of the gel forming reaction, and the support member containing the mixture of monomer, cross-linking agent and photoinitiator is subjected  
5 to U.V. irradiation at wavelengths of from 250 nm to 400 nm, for a period of a few seconds to a few hours. With certain photoinitiators, visible wavelength light may be used to initiate the polymerization. To permit the initiation, the support material must have a low absorbance at the  
10 wavelength used, to permit transmittance of the UV rays through the support. Preferably, the support and macroporous gel reagents are irradiated at 350nm for a few seconds to up to 2 hours.

Preferably, thermally initiated polymerization is carried  
15 out at 60-80°C for a few minutes up to 16 hours.

The rate at which polymerization is carried out has an effect on the size of the macropores obtained in the macroporous gel. As discussed earlier, when the concentration of cross-linker in a gel is increased to  
20 sufficient concentration, the constituents of the gel begin to aggregate to produce regions of high polymer density and regions with little or no polymer, which latter regions are referred to as "macropores" in the present specification. It is this mechanism which is affected by the rate of  
25 polymerization. When polymerization is carried out slowly, such as when a low light intensity in the photopolymerization, the aggregation of the gel constituents has more time to take place, which leads to larger pores in the gel. Alternatively, when the polymerization is carried  
30 out at a high rate, such as when a high intensity light source is used, there is less time available for aggregation and smaller pores are produced.

Once the composite materials are prepared, they can be washed with various solvents to remove any unreacted components and any polymer or oligomers that are not anchored within the support. Solvents suitable for the washing of the composite material include water, acetone, methanol, ethanol, and DMF.

#### *Uses of the Composite Material*

The composite material of the invention can find use in many different applications, where a liquid is passed through the macropores of the gel. The liquid passed through the macropores can be selected from, for example, a solution or a suspension, such as a suspension of cells or a suspension of aggregates.

In some embodiments, the composite materials can be used to perform separations. Uses include size exclusion separations such as ultrafiltration and microfiltration systems. The composite material of the invention is advantageous in these types of applications because of the wide range of pore-sizes available, and the ease with which composite materials with different pore-sizes can be made. In some embodiments, it is preferred that the composite materials used for size-exclusion separations are not fully occupied, i.e. that while all or substantially all the liquid that flows through the composite material flows through the macroporous gel, the void volume of the support member is not completely occupied by the macroporous gel. Composite materials having a non-fully occupied void volume where the density of the macroporous gel is greater at or adjacent to a first major surface of the support member than the density at or adjacent to a second major surface of the support member are referred to as being asymmetric.

In those embodiments where the macroporous gel bears a charge, the combination of macropore surface charge and of controlled macropore size produces a composite material that can be used in Donnan type exclusion separations. In these instances composite materials are produced that have high charge densities coupled with high hydraulic flows (flux). The high charge densities, coupled with high permeability, can be useful, for example, in adsorption of metal ions by giving composite materials with high ion-exchange capacities. The examples will demonstrate that these composite materials can also be used for the recovery of proteins and other related molecules, and that the composite materials of the invention exhibit high binding capacities.

Composite materials of the invention are also suitable for the separation of biomolecules, such as proteins, from solution, as the biomolecules may have specific interactions with ligands or binding sites found in the macropores of the composite materials. The specific interactions may involve electrostatic interactions, affinity interactions or hydrophobic interactions. Examples of molecules or ions, including biological molecules or ions, that can be separated include proteins such as albumins, e.g., bovine serum albumin, and lysozyme, but they can also be used in the separation of supramolecular assemblies such as viruses and cells. Examples of other biomolecules that can be separated include  $\gamma$ -globulins of human and animal origins, immunoglobulins such as IgG, IgM, or IgE of both human and animal origins, proteins of recombinant or natural origin including protein A, polypeptides of synthetic or natural origin, interleukin-2 and its receptor, enzymes such as phosphatase, dehydrogenase, etc., monoclonal antibodies, trypsin and its inhibitor, albumins of different origins, e.g., human serum albumin, chicken egg albumin, etc.,

cytochrome C, immunoglobulins, myoglobin, recombinant human interleukin, recombinant fusion protein, nucleic acid derived products, DNA and RNA of either synthetic or natural origin, and natural products including small molecules.

5 Biomolecule separations occur mostly in the macropores of the gel, but they can also take place, albeit at a slower rate, within the gel itself.

Some composite materials can be used as a reversible adsorbent. In these embodiments, a substance, for example a  
10 biomolecule, that is adsorbed in the macropores or in the mesh (micropore) of the gel can be released by changing the liquid that flows through the macroporous gel. Variations in the gel composition can be used to control the properties of the gel in terms of uptake and release of adsorbed  
15 substances. Another advantage of the composite material of the invention is that it can be made in the form of a membrane, and membrane-based biomolecule recovery is easier to scale up, less labor intensive, more rapid, and has lower capital costs than the commonly used conventional packed  
20 column chromatography techniques.

Some composite materials can also be used as solid supports for chemical synthesis or for cell growth. The reactants or nutrients required for these processes can either  
continuously flow through the macropores of the composite  
25 material, or they can be left to reside inside the macropores and then pushed out at a later time. In one embodiment, composite materials can be used in the stepwise production of peptides. In such an application, amino acids are attached to the macropore surface, and amino acid  
30 solutions are sequentially passed through the macropores to prepare peptide chains. The formed peptide can then be cleaved from the support by passing a suitable solvent

through the macropores. Supports currently used to carry out this type of synthesis consist of beads with small pores that offer slow diffusion characteristics. The composite materials of the invention have a more uniform pore structure, and they have through pores wherein the flow of liquids is not controlled diffusion.

Application areas include but are not limited to pharmaceuticals, including biotechnology, food, beverage, fine chemicals, and the recovery of metal ions.

#### 10 *Uses of Responsive Composite Materials*

The composite materials that comprise responsive macroporous gels (responsive composite materials) are preferably used to fractionate fluid components based on a size-exclusion mechanism. By this mechanism, steric hindrance is exerted on the convection and diffusion of a molecule or particle approaching the pores of a filtering device. The impediment to the transport is related to the ratio of the pore radius in the filter to the radius of the molecule or particle. When this ratio approaches to 1:1, molecules or particles will be completely retained by the filtering device. By changing the pore radius, molecules or particles of different sizes are permitted to pass through the pore and fractionation by size is achieved.

The responsive composite materials are well adapted to size exclusion mechanisms as the pores of these materials have a narrow pore-size distribution. This means that size exclusion separations can be achieved for molecules that have molecular weights that are much closer than normally permitted by conventional ultrafiltration/microfiltration membranes. In some embodiments, the size difference between the molecules being separated by the responsive composite

materials of the invention can be as low as about 0.9 nm. In other embodiments, the resolution is as low as about 0.5 nm. While some of the known ultrafiltration membranes do have narrow pore-size distributions (e.g. track etched  
5 membranes), these membranes have the disadvantage of being costly and of having relatively low fluxes.

In those embodiments where the composite materials are hydrophilic, the composite materials are well adapted to the size-separation of proteins as there is little or no  
10 adsorption of molecules to the composite material.

Normally, proteins are prone to non-specific binding. Such responsive composite materials are thus suitable for the separation of a multi-component protein mixture into discrete fractions based only on the size of the protein  
15 fractions. This separation of proteins through an exclusively size-exclusion method differs from the methods known in the art for separating proteins, where differences in size are used in conjunction with other physicochemical effects such as electrostatic charge, electrostatic double  
20 layer, and hydrophobic interaction effects, which physiochemical effects can lead to non-specific binding or even denaturation of the protein. Use of the composite material to separate proteins on a size-exclusion basis thus avoids the denaturation of the protein or its loss due to  
25 irreversible binding. The separation of proteins with the responsive composite material of the invention is a very gentle process that does not involve strong interactions between the protein and the separation medium, which permits the recovery of proteins with higher overall efficiencies, a  
30 major factor in the economics of therapeutic protein recovery.

One example of separations using the responsive composite materials is the separation of human serum albumin (HSA) (size of 60 kDa) from human immunoglobulin G (IgG) (size of 160 kDa). Size based separations of proteins are possible at fixed environmental conditions. With the responsive macroporous gel in a swollen or partially swollen state, e.g., at a low salt concentration in the feed with gels having weak acid or base functionalities, the protein with the lower molecular weight (e.g., human serum albumin) can be freely transmitted through the composite material, while the protein with the higher molecular weight (e.g., human immunoglobulin G) can be retained. When operated at a fixed environmental condition, the ultrafiltration process generates two product streams, and the fixed environment mode is suitable for fractionating binary mixtures, i.e. one protein from another.

The responsive composite materials of the invention can also be used to generate more than two product streams through size-based separations utilising the dynamic pore-size capabilities of the responsive composite material. This multi-component separation is possible as the change in membrane pore-size in response to change in environmental condition is gradual. When operated in this mode (i.e. with change in environmental condition), the process is suitable for separating proteins from a multi-protein mixture. In such a process, the environment is changed either in a stepwise fashion or gradually by appropriately altering the environment of the responsive gel. For example, changing the pH of the bulk medium used to carry out the filtration process using binary or ternary buffer systems can bring about a change in pore-size, and hence a sequential size based separation. When operated in the step change mode, each step will generate a fraction, each fraction containing

proteins smaller than in the next fraction. If  $n$  number of fractions are generated,  $(n-1)$  of these will be obtained in the permeate and the  $n^{\text{th}}$  fraction will be in the retentate.

The multi-component separations presented above represent a completely new use of ultra- and nano-filtration membranes. This type of multi-component separation is also referred to as chromatographic filtration. Some specific applications of this new type of separation include:

(a) fractionation of hen egg white components;

10           (i) LMW compounds such as Avidin (MW < 1000);

             (ii) Lysozyme (MW 14,100);

             (iii) Ovalbumin (MW 47,000);

             (iv) Conalbumin (MW 80,000);

(b) fractionation of human plasma proteins;

15           (i) Human serum albumin (HSA, MW 67,000);

             (ii) Human immunoglobulin G (HIgG, MW 155,00);

             (iv) Other human immunoglobulins (e.g. HIgM,  
MW >300,000);

(c) fractionation of dextran into molecular weight based  
20 fractions;

(d) fractionation of PEG into molecular weight based  
fractions;

(e) fractionation of polymers into molecular weight based  
fractions; and



(f) fractionation of micron-sized particles into size based fractions.

While the composite materials of the present invention are quite suitable for the separation of bulk materials, they  
5 can also be used to separate components on a smaller volume scale. For example, the responsive composite material can be used to separate biochemical substances such as antibodies, other bioactive proteins, hormones, polysaccharides and nucleic acids, prior to analysis. Many  
10 of the currently used biospecific analytical methods, e.g. Enzyme Linked Immuno Sorbent Assay (ELISA) are based on the binding of the above substances, or the binding of substances which biospecifically interact with these substances (e.g. antibodies, antigens, ligands, and  
15 substrate analogues), onto solid surfaces such as polystyrene (as in ELISA) or onto synthetic membranes (as in immuno-blotting). The detection limits of these tests are frequently limited by the surface area available in devices such as microwell plates or blotted sections of flat sheet  
20 membranes. Another limitation imposed by attachment of material onto solid surfaces is the likelihood of steric hindrance affecting the biospecific recognitions on which these tests are based. Biospecific analytical methods which rely on solution phase recognition and binding are also  
25 available, e.g. Radio Immuno Assay (RIA). These methods frequently rely on the use of porous synthetic membranes for retaining and enriching substances which are to be analyzed. However, the fixed nature of the permeability of these membranes could prove to be a limiting factor. The use of  
30 responsive composite materials would facilitate sequential removal of substance from solutions containing substances to be analyzed and thus facilitate analysis which would not be feasible with fixed permeability membranes. The responsive

membranes can also be utilised to facilitate removal from test solutions of substances which are likely to interfere with the assays.

While the responsive composite materials are especially  
5 suitable for use in size-exclusion separation, they can nonetheless be used in Donnan type separations and specific binding separations by the incorporation of appropriate monomers or polymers in the macroporous gel.

The responsiveness of composite material also permits the  
10 ability to open the pores of a membrane (made from the composite material) after use, and to then return and readjust the pores to their initial values by reversing the environmental change. Opening of the pores facilitates the cleaning of these membranes by removing a fouling material,  
15 thereby prolonging the effective use of the membrane.

#### EXAMPLES

The following examples are provided to illustrate the invention. It will be understood, however, that the specific details given in each example have been selected  
20 for purpose of illustration and are not to be construed as limiting the scope of the invention. Generally, the experiments were conducted under similar conditions unless noted.

#### EXPERIMENTAL

##### 25 *Materials Used*

The monomers used were acrylamide (AAM),  
2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS),  
(3-acrylamidopropane)trimethylammonium chloride (APTAC),  
diallyldimethylammonium chloride (DADMAC), ethylene glycol

dimethacrylate (EDMA), glycidyl methacrylate (GMA),  
N,N'-methylenebisacrylamide (BIS), methacrylic acid (MAA),  
acrylic acid (AA), and trimethylolpropane triacrylate  
(TRIM). The polymers used were branched poly(ethylene  
imine) (BPEI) of an average molecular weight (MW) of  
25000 Da, poly(ethylene glycol) (PEG) of average molecular  
weight of 200, 1000, 2000, 4000 and 10000 Da, and  
poly(allylammonium hydrochloride) (PAH) of an average  
molecular weight of 60000 Da. The cross-linker used for  
BPEI was ethylene glycol diglycidyl ether (EDGE).

The solvents used were cyclohexanol (CHX), methylene  
chloride ( $\text{CH}_2\text{Cl}_2$ ), deionized water, 1,4-dioxane,  
N,N-dimethylformamide (DMF), dodecanol (DDC), glycerol,  
methanol, 1-octanol, and 1-propanol.

The free radical polymerization initiators used were  
2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]2-hydroxy-2-  
methyl-1-propanone (Irgacure<sup>®</sup> 2959),  
2,2-dimethoxy-2-phenylacetophenone (DMPA), and  
1,1'-azobis(cyclohexanecarbonitrile) (VAZO<sup>®</sup> catalyst 88).

Proteins used were bovine serum albumin (BSA), lysozyme,  
human serum albumin (HSA) and human immunoglobulin (HIgG).

Other chemicals used were acryloyl chloride, hydrochloric  
acid, sodium azide, sodium chloride, sodium hydroxide,  
triethylamine, tris(hydroxymethyl)aminomethane (TRIS),  
4-morpholineethanesulfonic acid (MES) and buffers (Tris  
Buffer).

The porous supports used were poly(propylene) thermally  
induced phase separation (TIPS) membranes PP1545-4 with an  
average pore diameter of 0.45  $\mu\text{m}$ , thickness of 125  $\mu\text{m}$ , and  
porosity of 85 vol-%, produced by 3M Company, and PP1183-3X

of an average pore diameter of 0.9  $\mu\text{m}$ , thickness of 87  $\mu\text{m}$ , and porosity of 84 vol-%, both produced by 3M Company, and non-woven meltblown poly(propylene) TR2611A of a mean pore flow diameter of 6.5  $\mu\text{m}$ , thickness of 250  $\mu\text{m}$ , and porosity  
5 of 89.5 vol-% produced by Hollingworth & Vose Company.

#### *Preparation of Composite Materials*

The composite materials of the invention can be prepared according to the following general procedure. A weighed support member was placed on a poly(ethylene terephthalate)  
10 (PET) or poly(ethylene) (PE) sheet and a monomer or polymer solution was applied to the sample. The sample was subsequently covered with another PET or PE sheet and a rubber roller was run over the sandwich to remove excess solution. In situ gel formation in the sample was induced by polymerization  
15 initiated by irradiation with the wavelength of 350 nm for the period of 10 to 120 minutes or by heating the sandwich at 60-80°C for 2 hours. The irradiation was typically carried out using a system containing four 12" long lamps, approx. 1.5" spaced and emitting light at 365 nm with the  
20 output energy of approx. 0.1 Watt/inch. The system was equipped with a small fan to dissipate the heat (no other temperature control). The irradiated sample was located at approx. 5" distance from the lamps. In case when preformed polymer and in situ cross-linking was used to form the gel,  
25 the sandwich was left at room temperature until the cross-linking reaction was completed, typically for 2-16 hours. The resulting composite material was thoroughly washed with a suitable solvent or a sequence of solvents and stored in a 0.1 wt-% aqueous solution of sodium azide to prevent  
30 bacterial growth. In order to determine the amount of gel formed in the support, the sample was dried in vacuum at room temperature to a constant mass. The mass gain due to

gel incorporation was calculated as a ratio of an add on mass of the dry gel to the initial mass of the porous support.

#### *Flux Measurements*

5 Water flux measurements through the composite materials were carried out after the samples had been washed with water. As a standard procedure, a sample in the form of a disk of diameter 7.8 cm was mounted on a sintered grid of 3-5 mm thickness and assembled into a cell supplied with compressed  
10 nitrogen at a controlled pressure. The cell was filled with deionized water or another feed solution and a desired pressure was applied. The water that passed through the composite material in a specified time was collected in a pre-weighed container and weighed. All experiments were  
15 carried out at room temperature and at atmospheric pressure at the permeate outlet. Each measurement was repeated three or more times to achieve a reproducibility of  $\pm 5\%$ .

The water flux,  $Q_{H_2O}$  (kg/m<sup>2</sup>h), was calculated from the following relationship:

$$Q_{H_2O} = \frac{(m_1 - m_2)}{A \cdot t}$$

20 where  $m_1$  is the mass of container with the water sample,  $m_2$  is the mass of container,  $A$  is the active membrane surface area (38.5 cm<sup>2</sup>) and  $t$  is the time.

The composite material of the invention may have water flux values that are smaller than those of the unfilled support  
25 member, with possible flux reduction of about a factor of two to about of a factor of a few hundred depending on the

application. For ultrafiltration application, the flux may be reduced by a factor of about ten to about a few hundred.

The hydrodynamic Darcy permeability,  $k$  ( $\text{m}^2$ ) of the membrane was calculated from the following equation

$$k = \frac{Q_{H_2O} \eta \delta}{3600 d_{H_2O} \Delta P}$$

where  $\eta$  is the water viscosity ( $\text{Pa}\cdot\text{s}$ ),  $\delta$  is the membrane thickness (m),  $d_{H_2O}$  is the water density ( $\text{kg}/\text{m}^3$ ), and  $\Delta P$  (Pa) is the pressure difference at which the flux,  $Q_{H_2O}$ , was measured.

- 10 The hydrodynamic Darcy permeability of the membrane was used to estimate an average hydrodynamic radius of the pores in the porous gel. The hydrodynamic radius,  $r_h$ , is defined as the ratio of the pore volume to the pore wetted surface area and can be obtained from the Carman-Kozeny equation given in  
15 the book by J. Happel and H. Brenner, Low Reynolds Number Hydrodynamics, Noordhof Int. Publ., Leyden, 1973, p. 393:

$$k = \frac{\epsilon r_h^2}{K}$$

where  $K$  is the Kozeny constant and  $\epsilon$  is the membrane porosity. The Kozeny constant  $K \approx 5$  for porosity  $0.5 < \epsilon < 0.7$ .

- 20 The porosity of the membrane was estimated from porosity of the support by subtracting the volume of the gel polymer.

#### *Protein adsorption/desorption experiment*

Protein adsorption experiments were carried out with two proteins, namely, bovine serum albumin (BSA) and lysozyme.

- 25 In the case of experiments with a positively charged composite material in the form of a membrane, the membrane

sample was first washed with distilled water and subsequently with a TRIS-buffer solution (pH=7.8). In an adsorption step, a composite material sample in a form of a single membrane disk of diameter 7.8 cm was mounted on a sintered grid of 3-5 mm thickness in a cell used for water flux measurements and described above. A BSA solution, comprising from 0.4 to 0.5 mg BSA per ml of buffer solution, was poured to the cell to give a 5 cm head over the composite material. This hydrostatic pressure of 5 cm was kept constant by further additions of the BSA solution. In a modification of this method, the cell was pressurised with compressed nitrogen. The flow rate was measured by weighing the amount of permeate as a function of time. Typical values varied between 1 and 5 ml/min. Permeate samples were collected at 2-5 min intervals and analyzed by UV analysis at 280 nm. Following the adsorption step, the composite material in the cell was washed with about 200 ml of the TRIS-buffer solution, and desorption was carried out with a TRIS-buffer solution containing 1M NaCl at 5 cm head pressure or under a controlled pressure of compressed nitrogen. The permeate samples were collected at 2-5 min intervals and tested by UV analysis at 280 nm for BSA content.

For negatively charged composite materials, a solution of lysozyme in a MES buffer solution having a pH of 5.5 and a lysozyme concentration of 0.5g/L was used in a procedure similar to that described above for BSA and positively charged materials. The flow rate during the protein adsorption was again kept within 1-5 ml/min. Prior to the desorption of the protein, the membrane was washed by passing with 200 ml of the buffer solution. The desorption of the protein was carried out using a MES buffer solution (pH = 5.5) containing 1M NaCl in the same way as described

above for the desorption of BSA. The lysozyme content in the collected samples was determined by UV spectrophotometry at 280 nm.

In other examples, protein adsorption tests involve stacks of several membranes of diameter of 19 mm mounted into a Mustang® Coin Device manufactured by Pall Corporation and the protein solution was delivered to the membrane stack at controlled flow rate using a peristaltic pump. The permeate fractions were collected and analyzed in the same way as described above. The desorption of the proteins was carried in a similar way as described above, with buffered 1M NaCl delivered to the membrane stack by using the pump instead of gravity or compressed nitrogen pressure.

#### *Protein separation experiment*

The experimental method used to examine the separation properties of the responsive composite materials of this invention in protein-protein fractionation processes is based on the pulsed injection ultrafiltration technique and its derivatives developed by Ghosh and his co-workers and described in the following articles: R.Ghosh and Z.F. Cui, Analysis of protein transport and polarization through membranes using pulsed sample injection technique, Journal of Membrane Science, vol. 175, no. 1 (2000) p. 75 - 84; R.Ghosh, Fractionation of biological macromolecules using carrier phase ultrafiltration, Biotechnology and Bioengineering, vol. 74, no. 1 (2001) p. 1 - 11; and R. Ghosh, Y. Wan, Z.F. Cui and G. Hale, Parameter scanning ultrafiltration: rapid optimisation of protein separation, Biotechnology and Bioengineering, vol. 81 (2003) p. 673-682 and incorporated herein by reference. The experimental set-up used was similar to that used for parameter scanning



ultrafiltration as described in article by R. Ghosh, Y. Wan, Z.F. Cui and G. Hale, Parameter scanning ultrafiltration: rapid optimisation of protein separation, Biotechnology and Bioengineering, vol. 81 (2003) p. 673-682.

5 A binary carrier phase system was used in the ultrafiltration experiments. The starting carrier phase in all the responsive composite material experiments was one with a low salt concentration (typically 5-10 mM NaCl). In all these experiments the carried phase was switched to one  
10 with a high salt concentration (typically 1 M NaCl). The change in salt concentration within the membrane module could be tracked by observing the conductivity of the permeate stream. The change in transmembrane pressure gave an idea about the change in membrane hydraulic permeability  
15 with change in salt concentration.

#### Example 1

This example illustrates the formation of an unsupported porous gel, which can be used as the macroporous gel to prepare the composite material of the invention.

20 A solution containing 3.33 g of (3-acrylamidopropane)trimethylammonium chloride (APTAC) monomer as a 75% aqueous solution, 0.373 g of *N,N'*-methylenebisacrylamide (BIS) cross-linker, and 0.0325 g of Irgacure @2959 photoinitiator dissolved in 25 ml of a  
25 dioxane:dimethylformamide:water mixture, with the solvent volume ratio of 71:12:17, respectively, was prepared. In this solvent mixture, dioxane is a poor solvent while DMF and water are good solvents. A total monomer concentration (APTAC and BIS) of 0.58 mol/L was thus obtained. The cross-  
30 linking degree was 20 mol %, based on APTAC. 5 ml of this solution was placed in a glass vial and subjected to UV

irradiation at 350nm for 2hrs. A white gel was formed which was washed thoroughly with de-ionized water to exchange the reaction solvent and remove the unreacted monomer or soluble oligomers.

- 5 The gel formed was mechanically very weak. A sample of the gel was examined using an environmental scanning electron microscope (ESEM) with water vapor present in the sample chamber to prevent drying of the gel. The micrograph, shown in Figure 1, has dark, cavernous areas that indicate that a  
10 macroporous gel was formed.

#### Example 2

This example illustrates a method of preparing a positively charged composite material of the present invention using the monomer solution of composition described in example 1  
15 applied to a sample of the poly(propylene) porous support PP1545-4. The composite material was prepared according to the general procedure described above using UV irradiation at 350 nm for 2 hours. After polymerization, the composite material was washed with with de-ionized water for 48hrs.

- 20 Mass gain of the resulting composite material after drying was 107 wt%, water flux was  $1643 \pm 5$  kg/m<sup>2</sup>h at 50kPa and Darcy permeability was  $9.53 \times 10^{-16}$  m<sup>2</sup>.

The morphology of the gel-incorporated composite material was examined using ESEM in the same manner as described in  
25 Example 1. The ESEM micrograph shown in Figure 2 shows that the macroporous gel has been incorporated into the host membrane. The micrograph shows a similar structure to that of the unsupported macroporous gel shown in Figure 1 and little evidence of the microporous support member.

## Example 3

This example illustrates a method of preparing a negatively charged composite material of the present invention, with a weak acid functionality.

5 5.50 g of vacuum-distilled methacrylic acid (MAA) monomer, 0.4925 g of *N,N'*-methylenebisacrylamide cross-linker and 0.1503 g of Irgacure® 2959 photoinitiator were dissolved in 25 ml of a dioxane:DMF solvent mixture with a volume ratio of 9:1, respectively, to prepare the starting monomer  
10 solution. The composite material was prepared using the poly(propylene) PP1545-4 support and the general procedure for the photoinitiated polymerization described above. The irradiation time used was 2 hours and the resulting membrane was washed with DMF for 24 hrs followed by a 48 hr wash with  
15 deionized water. The mass gain of the resulting dried membrane was 231 wt%, water flux was  $4276 \pm 40$  kg/m<sup>2</sup>h at 50kPa and Darcy permeability was  $2.64 \times 10^{-15}$  m<sup>2</sup>.

The protein (lysozyme) absorption/desorption characteristics of the composite material were examined using the general  
20 procedure for a single membrane disk outlined earlier. The concentration of the protein used in this experiment was 0.5 g/L in a 10 mM MES buffer at pH 5.5. The flow rate of adsorption experiment was regulated to be 2-4 ml/min. A plot of the concentration of lysozyme in permeate versus the  
25 volume of permeate is shown in Figure 3. It can be seen that even with the single membrane disk, a relatively steep break through curve is obtained indicating a uniform and narrow pore size distribution in the membrane. The composite material has a breakthrough lysozyme binding  
30 capacity of 42.8 mg/mL. A desorption experiment with a

buffer solution containing 1M NaCl indicated that the recovery of protein was 83.4%.

#### Example 4

This example illustrates the effect of the total monomer  
5 concentration and solvent mixture on the hydraulic flow rate  
(flux) of composite membranes with weak acid functionality  
of the type described in Example 3.

A series of composite membranes (MAA1 through MAA5) were  
prepared using monomer solutions of chemical compositions  
10 listed in Table 1 and the porous support PP1545-4. The  
preparation procedure described in Example 3 was employed.

Table 1: The effect of the total monomer concentration and solvent mixture on water flux of composite membranes

Sample I.D.	Total Monomer Concentration (MAA + BIS) (mol/L)	Cross-linking Degree (mol-%)	Solvent Mixture (volume part)		Mass Gain (wt%)	Flux at 50kPa (kg/m <sup>2</sup> .h)
			Dioxane	DMF		
MAA1	1.71	5	8	2	71	12.2±0.1
MAA2	2.19	5	8	2	153	94±14
MAA3	2.68	5	8	2	177	1265±111
MAA4	3.66	5	8	2	300	1800±9
MAA5	2.68	5	9	1	231	4276±40

As can be seen from Table 1, the hydraulic flow rate (flux) of composite membranes of the present invention can be tuned by adjusting the monomer loading in the solution. Contrary to the typical trends found with homogeneous gels, for which  
5 an increase in gel density is followed by decrease in permeability, the increase in the mass gain in the membranes of this series results in the flux increase. Further increase in flux is achieved when the concentration of the poor solvent (dioxane) in the solvent mixture is increased  
10 (compare samples MAA3 and MAA5).

#### Example 5

This example illustrates a method of preparing a negatively charged composite material of the present invention that has strong acid functionality.

15 A solution containing 2.50g 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) monomer, 0.372g N,N'-methylenebisacrylamide cross-linker and 0.0353g Irgacure® 2959 photo-initiator, dissolved in 25 ml of a dioxane:H<sub>2</sub>O mixture with a volume ratio 9:1, respectively, was used. A  
20 composite material was prepared from the solution and the support PP1545-4 using the photoinitiated polymerization according to the general procedure describe above. The irradiation time used was 1 hour at 350 nm. After polymerization, the membrane was extracted with de-ionized  
25 water for 48 hrs. The mass gain of the resulting membrane was 74.0 wt%, water flux was 2559±40 kg/m<sup>2</sup>h at 50 kPa and Darcy permeability was 1.58×10<sup>-15</sup> m<sup>2</sup>.

#### Example 6

This example illustrates further the effect of the solvent  
30 mixture composition and the cross-linking degree on the

hydraulic flow rate of composite membranes with the strong acid functionality. A series of composite membranes (AMPS1 through AMPS5) was prepared using chemical compositions listed in Table 2 following the general preparation  
 5 procedure and the irradiation conditions as in example 5.

Table 2: The effect of solvent mixture on water flux of the composite membranes

Sample I.D.	Total Monomer Conc. (mol/L)	Cross-linking Degree (mol%)	Solvent mixture (volume part)			Mass gain (wt%)	Flux at 50 kPa (kg/m <sup>2</sup> h)
			Dioxane	DMF	H <sub>2</sub> O		
AMPS1	0.48	20	5	5	0	92	3.2±0.0
AMPS2	0.48	20	8	2	0	100	575±12
AMPS3*	0.48	20	9	0	1	74	2559±9
AMPS4	0.48	10	8	2	0	100	8.4±0.0

AMPS3 is the composite membrane prepared in the previous  
 10 Example.

As can be seen, a similar pattern to that described in example 4 was observed with regards to the relationship between solubility of polymer in the solvent and water flux of composite membranes. Comparison of AMPS2 with AMPS 4  
 15 shows that hydraulic flow rate (flux) of a composite membrane can also be adjusted by the degree of cross-linking.

## Example 7

This example illustrates the effect of introducing a neutral co-monomer into a negatively charged composite material of the present invention.

- 5 A solution containing 1.750 g of 2-acrylamido-2-methyl-1-propanesulfonic acid, 0.485 g of acrylamide, 0.868 g of N,N'-methylenebisacrylamide cross-linker, and 0.044 g of Irgacure® 2959 photo-initiator, dissolved in 25 ml of a dioxane:DMF:H<sub>2</sub>O mixture with a volume ratio 8:1:1,  
10 respectively, was prepared. A composite material was prepared from the solution and the support PP1545-4 using the photoinitiated polymerization according to the general procedure describe above. The irradiation time used was 1 hour at 350 nm. After polymerization, the membrane was  
15 extracted with de-ionized water for 48hrs.

The mass gain of the resulting membrane was 103 wt%, water flux was  $7132 \pm 73$  kg/m<sup>2</sup>.h at 100kPa, and Darcy permeability was  $4.40 \times 10^{-15}$  m<sup>2</sup>.

## Example 8

- 20 This example illustrates one method of making a positively charged composite material of this invention.

A 15 wt-% solution was prepared by dissolving diallyldimethylammonium chloride (DADMAC) monomer and N,N'-methylenebisacrylamide (BIS) cross-linker in a molar  
25 ratio of 5:1, respectively, in a solvent mixture containing 37 wt-% water, 45 wt-% dioxane and 18 wt-% DMF. The photo-initiator Irgacure® 2959 was added in the amount of 1% with respect to the mass of the monomers.



A composite material was prepared from the solution and the support PP1545-4 using the photoinitiated polymerization according to the general procedure describe above. The irradiation time used was 30 minutes at 350 nm. The  
5 composite material was removed from between the polyethylene sheets, washed with water and TRIS-buffer solution and stored in water for 24 hrs.

Several samples similar to that described above were prepared and averaged to estimate the mass gain of the  
10 composite material. The substrate gained 42.2 % of the original weight in this treatment.

The composite material produced by this method had a water flux in the range of 2100-2300 kg/m<sup>2</sup> hr at 70 kPa and Darcy permeability of  $9.87 \times 10^{-16}$  m<sup>2</sup>.

15 The protein (BSA) adsorption characteristic of the composite material was examined using the general procedure for a single membrane disk described above. The concentration of the protein used in this experiment was 0.4 g/L in 50 mM TRIS-buffer. The flow rate was 2-4 ml/min. A plot of the  
20 concentration of BSA in the permeate vs. the permeate volume is shown in Figure 4. The composite material had a BSA binding capacity of 48-51 mg/ml. The BSA desorption was found to be in the range of 78-85%.

#### Example 9

25 This example illustrates that by adding a neutral monomer to the charged monomer used in Example 6 the protein binding capacity can be substantially increased.

A 10 wt-% solution was prepared by dissolving diallyldimethylammonium chloride (DADMAC) and acrylamide

(AAM), in the ratio 80:20, in a solvent mixture containing 63 wt-% dioxane, 18 wt-% water, 15 wt-% DMF, and 4 wt-% dodecanol. N,N'-methylenebisacrylamide cross-linker was added to the monomer solution to obtain 40% (mol/mol) cross-  
5 linking degree. The photoinitiator Irgacure® 2959 was added in the amount of 1% with respect to the total mass of monomers.

A composite material was prepared from the solution and the support PP1545-4 using the photoinitiated polymerization  
10 according to the general procedure describe above. The irradiation time used was 20 minutes at 350 nm. The composite material was removed from between the polyethylene sheets, washed with water, TRIS-buffer solution and stored in water for 24 hrs.

15 A similar sample to that described above was prepared and used to estimate the mass gain of the composite material. The substrate gained 80 % of the original weight in this treatment.

The composite material produced by this method had a water  
20 flux in the range of 250 kg/m<sup>2</sup> hr at 70 kPa and Darcy permeability was  $1.09 \times 10^{-16}$  m<sup>2</sup>.

The protein (BSA) adsorption characteristic of the composite material was examined using the general procedure for a single membrane disk described above. The protein  
25 concentration was 0.4 g/L in a 50 mM TRIS buffer solution. The flow rate of absorption experiment was adjusted to 2-4 ml/min. The composite material had a BSA binding capacity of 104 mg/ml.

## Example 10

This example illustrates the formation of a supported porous gel composite material by cross-linking of a pre-formed polymer.

5 Three separate solutions were prepared with the following compositions: (A) 20 g of branched poly(ethyleneimine) (BPEI) (25,000 Da) in 50 ml of methanol, (B) 20 g of poly(ethyleneglycol) PEG (~10,000 Da) in 50 ml of methanol, and (C) ethyleneglycol diglycidyl ether (0.324 g) in 5 ml  
10 of methanol.

A mixture of the three solutions was prepared consisting of 2 ml of (A), 3 ml of (B), and 5 ml of (C). A portion of this resulting solution was allowed to stand in a vial overnight when a phase separation was observed. Examination  
15 of the morphology of the upper clear gel layer indicated that it was macroporous.

The same mixed solution was spread on a sample of poly(propylene) support PP1545-4 using the techniques described in the general procedure. The membrane was  
20 sandwiched between two poly(ethyleneterephthalate) sheets and allowed to stand overnight. The composite material was extracted with methanol at room temperature for 24 h, and a mass gain of 95% was observed. The water flux of the composite material was  $6194 \text{ kg/m}^2 \text{ h}$  at 100 kPa and Darcy  
25 permeability was  $4.89 \times 10^{-10} \text{ m}^2$ .

The dynamic protein absorption capacity of the composite material was measured using a BSA solution (0.4 mg/mL) in the method for a single membrane disk described in the general section above. It had a capacity of 68 mg/ml before  
30 breakthrough.

## Example 11

This example illustrates the effect of monomer mixture composition and the polymerization conditions on the hydraulic properties of composite materials prepared by *in situ* polymerization of glycidyl methacrylate (GMA) with ethylene dimethacrylate (EDMA) used as a cross-linker. The solvents used were dodecanol (DDC), cyclohexanol (CHX), and methanol. A porous polypropylene support membrane PP1545-4 and two modes of initiation of *in situ* polymerization were used according to the general procedure described above. In the photopolymerization mode, 2,2-dimethoxy-2-phenylacetophenone (DMPA) was used as a photoinitiator while the thermal polymerization was initiated by 1,1'-azobis(cyclohexanecarbonitrile). In both modes, the polymerization was carried out for 2 hours.

The polymerization conditions and properties of the composite materials containing porous poly(glycidyl methacrylate-co-ethylene diacrylate) are presented in Table 3.

Table 3. Porous poly(glycidyl methacrylate-co-ethylene diacrylate)-filled composite materials

Membrane ID	Total Monomer Concentration wt-%	Solvent	Initiation Mode of Polymerization	Mass Gain wt-%	Darcy Permeability m <sup>2</sup>	Hydrodynamic radius nm
AM612	43.8	DDC/CHX 9/91	Photo	276.8	$6.96 \times 10^{-16}$	95.1
AM614	22.9	DDC/CHX 9/91	Photo	144.3	$2.77 \times 10^{-15}$	148.5
AM615	47.6	DDC/CHX 9/91	Thermal	237.0	$1.66 \times 10^{-17}$	13.0
AM616	24.9	DDC/CHX 9/91	Thermal	157.5	$9.15 \times 10^{-16}$	86.4
AM619	48.6	Methanol	Photo	265.0	$2.48 \times 10^{-15}$	163.8

The mass gain obtained in this series of composite materials is proportional to the total monomer concentration in the polymerization mixture. Membranes AM612, AM615, and AM619 were prepared using high concentration of monomers while in  
5 membranes AM614 and AM616 the monomer concentration was cut approximately by half (Table 3).

The high values of the pure water flux measured at 100 kPa of transmembrane pressure (Table 3) indicate that the pore-filling material is macroporous. The pure water flux and,  
10 consequently, the hydraulic radius are affected not only by the mass gain but also by the polymerization mode. As shown in Figure 5, the hydraulic radius is a linear function of the mass gain with the slope depending on the polymerization mode. The absolute value of the negative  
15 slope in the thermal polymerization is twice that of the photopolymerized composite materials. This means that photopolymerized composite materials have larger pores than that of the thermally polymerized ones at the same mass gains. Thus, the photo-initiated polymerization, which is  
20 faster than the thermally-initiated one, produces larger pores. Since the monomer conversion is practically the same in both cases of polymerization (similar mass gains), the presence of the poly(propylene) substrate either through its hydrophobic nature or by creating microscopic confinements  
25 for the polymerization affects the pore formation and the final structure of the pore-filling materials.

By changing the solvents from dodecanol/cyclohexanol 9/91 to methanol, which is cheaper and environmentally more acceptable than the other solvents, a composite material  
30 with very high flux was obtained (membrane AM619, Table 3). The composite material was produced from the concentrated monomer mixture and had flux comparable with that of the

membrane AM614 which had a mass gain almost twice as low as that of AM619.

This and subsequent examples illustrate a feature of some composite materials of the invention. With the capability  
5 to change the composition and concentration of the monomers and solvents, there can be produced stable composite materials with different porous structures. As shown in Table 3, composite materials with larger pores can be made in this way.

#### 10 Example 12

This example illustrates further the effect of monomer mixture composition on the hydraulic properties of composite materials of this invention.

A series of composite materials have been prepared according  
15 to the general procedure described above and containing porous poly(acrylamide) gels formed by *in situ* photoinitiated polymerization of acrylamide (AAM) and *N,N'*-methylenebisacrylamide (BIS) as a cross-linker in the pores of a poly(propylene) support membrane. The porous support  
20 member used was poly(propylene) TIPS membrane PP1545-4. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) or 1-[4-(2-hydroxyethoxy)phenyl]2-hydroxy-2-methyl-1-propane-1-one (Irgacure<sup>®</sup> 2959) were used as photoinitiators. Irradiation  
25 was carried out at 350 nm for 2 hours. Composition of the pore-filling solutions and the properties of the resulting composite materials are summarized in Table 4.

Table 4. Composition and properties of poly(acrylamide)-filled composite materials

Membrane I.D.	Degree of XL Wt-%	Total Monomer Conc. wt-%	Solvent 1		Solvent 2		Mass Gain %	Darcy Permeability $\text{m}^2$	Hydrodynamic Radius nm
			Name	Conc. wt-%	Name	Conc. wt-%			
AM606	18.0	13.3	Water	86.6	None	0.0	111.7	$9.3 \times 10^{-18}$	8.0
AM607	18.0	13.6	Water	67.9	Methanol	18.4	107.8	$2.5 \times 10^{-18}$	4.1
AM608	18.0	14.4	Water	71.8	Glycerol	13.7	110.4	$2.1 \times 10^{-18}$	3.8
AM609	16.8	12.0	Water	51.9	Glycerol	36.0	103.3	$1.6 \times 10^{-18}$	3.3
AM610	31.8	34.5	DMF	49.1	1-Propanol	16.4	307.2	$3.9 \times 10^{-17}$	20.0
AM611	32.0	18.7	DMF	60.9	1-Propanol	20.5	130.3	$5.3 \times 10^{-16}$	62.7
AM617	32.2	34.9	DMF	48.4	1-Octanol	16.7	273.3	$8.6 \times 10^{-17}$	29.0



Membranes AM606 through AM609 have been prepared using very similar concentration of monomers (12.0-14.4 wt-%) and a similar, relatively high, degree of cross-linking (16.8-18.0 wt-% of monomers). The mass gains obtained with these composite materials are also very similar. As shown in Figure 6, there is a linear relationship between total monomer concentration in the pore-filling solution and the mass gain achieved after photopolymerization.

The high degree of cross-linking in the composite material prepared from aqueous solution without non-solvent (AM606) leads to relatively high permeability. Surprisingly, the addition of methanol or glycerol, which are poor solvents for linear poly(acrylamide), to water, which is a good solvent for the linear polymer, brings about a substantial reduction in the Darcy permeability and the hydrodynamic radius calculated on its basis. The reduction in permeability is higher with glycerol than with methanol and increases with the amount of glycerol in the solution.

The use of mixtures of poor solvents, such as *N,N'*-dimethylformamide and 1-propanol or 1-octanol, as well as the further increase of the degree of cross-linking and total monomer concentration have been tested in membranes AM610, AM611, and AM617. As shown in Table 4, substantially higher permeabilities and hydraulic radii are obtained with all these composite materials as compared to the composite materials prepared with water as one of the solvents. This occurred despite an increase of the total monomer concentration; more than double in membranes AM610 and AM617 than that in the membranes prepared with water as one of the solvents. Changing the other solvent from 1-propanol in AM610 to 1-octanol in AM617 also brings about substantial increase in permeability and hydraulic radius.

Microscopic images of the surface of membrane AM610 are shown in Figures 7 (AFM) and 8 (ESEM). For comparison, an ESEM image of the nascent porous support member is also shown in Fig. 8. Both sets of images show a porous phase-separated gel covering the member surface with no discernible elements of the support member.

Membrane AM611 was prepared with DMF and 1-propanol but the total monomer concentration was just over half that of AM610. Membrane AM611 shows very high flux and the hydraulic radius three times that of AM610. The ESEM images of the surface of this membrane are presented in Figure 9. It shows a highly porous gel structure (top image) that resembles the bulk gel formed in some spots on the membrane surface but detached from the membrane (bottom picture).

A comparison of surfaces of membranes AM610 and AM611 is presented in Figure 10. The large difference in the size of the structural elements in these two gels is clearly visible.

The composite materials prepared in this example can serve as ultrafiltration membranes. It has been shown that the pore size of the composite material and, therefore, its separation properties, can be controlled to achieve a wide range of values.

#### Example 13

This example illustrates the effect of pore size of the support member on the hydraulic flow rate (flux) through composite materials of this invention.

Two polypropylene support membranes of pore size 0.45  $\mu\text{m}$  and 0.9  $\mu\text{m}$ , PP1545-4 and PP1183-3X, respectively, were used to

produce composite materials with the same monomer mixture containing 39.4 wt-% of glycidyl methacrylate and 9.2 wt-% of ethylene diacrylate in methanol, thus having 48.6 wt-% of monomers and 18.9 wt-% of ethylene diacrylate (cross-linker) in the monomer mixture. The photoinitiator used was DMPA in the amount of 1.3 wt-% of monomers.

The composite materials were prepared according to the general procedure described above. The irradiation time was 2 hours at 350 nm. The resulting composite materials were washed with methanol followed by deionized water. The composite materials were tested for water flux at 100 kPa to calculate the Darcy permeability and hydraulic radius. The results are presented in Table 5.

Table 5. Hydraulic properties of composite membranes produced with substrates of different pore sizes

Membrane ID	Support pore size ( $\mu\text{m}$ )	Mass Gain (wt-%)	Flux at 100 kPa ( $\text{kg}/\text{m}^2\text{h}$ )	Hydrodynamic radius (nm)	Average hydrodynamic radius (nm)	Standard Deviation (%)
AM619	0.45	265.0	8080.9	163.8	156.2	6.9
AM620	0.90	296.8	7310.1	148.5		

The data show that the hydraulic radius in both composite materials is the same within an experimental error, proving that the composite materials contain macroporous gels of similar structure.

#### 5 Example 14

This Example illustrates the synthesis of poly(ethylene glycol) (PEG, MW's 4000, 2000, 1,000, and 200) diacrylates, which can be used as cross-linkers to prepare the composite material of the invention.

10 The synthesis procedure used follows that described by N. Ch. Padmavathi, P. R. Chatterji, *Macromolecules*, 1996, 29, 1976, which is incorporated herein by reference. 40g of PEG 4000 was dissolved in 150ml of  $\text{CH}_2\text{Cl}_2$  in a 250-ml round bottom flask. 2.02g of triethylamine and 3.64g of acryloyl  
15 chloride were added dropwise to the flask separately.

Initially the reaction temperature was controlled at  $0^\circ\text{C}$  with an ice bath for 3hrs, and then the reaction was allowed to warm to room temperature and kept for 12 hrs. The reaction mixture was filtered to remove the precipitated  
20 triethylamine hydrochloride salt. The filtrate then was poured into an excess of n-hexane. The colorless product, referred to as PEG 4000 diacrylate, was obtained by filtration and drying at room temperature.

The same procedure was used with the PEG's of other  
25 molecular weights. The molar ratios of the PEG to acryloyl chloride were kept the same as used above with PEG 4000.

## Example 15

This example illustrates a further method of preparing a negatively charged composite material that has a high adsorption capacity for lysozyme.

- 5 A solution containing 0.6g of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), and 0.4g of acrylamide (AAM) as monomers, 0.25g of *N,N'*-methylenebisacrylamide (BIS) and 1.0g of PEG 4000 diacrylate obtained in Example 14 as cross-linkers, and 0.01g of Iragure ®2959 as a photoinitiator was  
10 prepared in 10ml of solvent consisting of a 80:10:10 volume ratio of dioxane, dimethylformamide (DMF), and water.

A porous poly(propylene) support member in the form of a membrane (PP1545-4) was used and the composite material was prepared according to the general procedure, with the  
15 irradiation carried out at 350 nm for 20 minutes. After polymerization, the composite material was washed thoroughly with de-ionized water for 24hrs.

Mass gain of the resulting composite material after drying was 113.2wt%, water flux was  $366 \pm 22$  kg/m<sup>2</sup>h at 100 kPa, and  
20 Darcy permeability was  $2.26 \times 10^{-16}$ .

The protein (lysozyme) absorption/desorption characteristics of the composite material were examined using the general procedure for a single membrane disk outlined earlier. The concentration of the protein used in this experiment was  
25 0.5 g/L in a 10mM MES buffer at pH 5.5. The flow rate of adsorption experiment was regulated to be 2-4ml/min. A plot of the concentration of lysozyme in the permeate versus the volume of permeate is shown in Figure 11. It can be seen that a relatively steep break through curve is obtained.  
30 The composite material had a Lysozyme binding capacity of

103.9 mg/ml. A desorption experiment indicated that the recovery of protein was 64.0%.

#### Example 16

This example illustrates preparation of a negatively charged composite material with the same nominal polymer composition as in Example 15 but with much higher hydraulic flows (flux) and good lysozyme uptake capacity.

The monomer solution was produced by dilution of the solution formulated in Example 15 with acetone with the mass ratio of 1:1.

A porous poly(propylene) support member in the form of a membrane (PP1545-4) was used and the composite material was prepared according to the general procedure. The irradiation time used was 2 hours. After polymerization, the composite material was washed thoroughly with de-ionized water for 24hrs.

The mass gain of the resulting composite material after drying was 51.1wt% and water flux was  $6039 \pm 111$  kg/m<sup>2</sup>.h at 100kPa giving Darcy permeability of  $3.73 \times 10^{-15}$ .

The protein (lysozyme) absorption/desorption characteristics of the composite material were examined using the general procedure for a single membrane disk outlined earlier. The concentration of the protein used in this experiment was 0.5 g/L in a 10 mM MES buffer at pH 5.5. The flow rate of adsorption experiment was regulated to be 2-4 ml/min. A plot of the concentration of lysozyme in permeate versus the volume of permeate is shown in Figure 12. It can be seen that a relatively steep break through curve is obtained. The composite material had a lysozyme binding capacity of

75.4 mg/ml. A desorption experiment indicated that the recovery of protein was 65.0%.

#### Example 17

This example illustrates a further preparation of a  
5 negatively charged composite material that has a very high flux but lower protein binding capacity.

The monomer solution was produced by dilution of the solution formulated in Example 15 with acetone with the mass ratio of 1:2.

10 A porous poly(propylene) support member in the form of a membrane (PP1545-4) was used and the preparation of composite material was carried out according to the general procedure described above. UV initiated polymerization was carried out for 2 hours. After polymerization, the composite  
15 material was washed thoroughly with de-ionized water for 24hrs.

The mass gain of the resulting composite material after drying was 34.4wt% and water flux was  $12184 \pm 305$  kg/m<sup>2</sup>h at 100 kPa giving Darcy permeability of  $7.52 \times 10^{-15}$ .

20 The protein (lysozyme) absorption/desorption characteristics of the composite material were examined using the general procedure for a single membrane disk outlined earlier. (The concentration of the protein used in this experiment was 0.5 g/L in a 10 mM MES buffer at pH 5.5. The flow rate of  
25 adsorption experiment was regulated to be 2-4 ml/min.) A plot of the concentration of lysozyme in permeate versus the volume of permeate is shown in Figure 13. It can be seen that a relatively steep break through curve is obtained. The composite material had a lysozyme binding capacity of



53.5 mg/ml. A desorption experiment indicated that the recovery of protein was 99.0%.

Examples 15, 16 and 17 show that it is possible to control the loading of porous gel into the host membrane thereby controlling the water flux at a defined pressure (100 kPa in the data given in the examples) and also that the lysozyme uptake is related to the mass of incorporated porous gel.

#### Example 18

This example illustrates preparation of a negatively charged membrane that has both good protein adsorption capacity and good flux using a macromonomer.

A monomer solution containing 0.6 g of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), 0.4 g of acrylamide (AAM), 0.25 g of *N,N'*-methylenebisacrylamide (BIS), 0.01 g of Irgacure® 2959, and 1.0 g of PEG 2000 macromonomer obtained in Example 14, dissolved in 10 ml of a dioxane-(DMF)-water mixture with a volume ratio 80:10:10, respectively, was prepared.

A microporous poly(propylene) support member in the form of a membrane, support PP1545-4, was used together with the general procedure described above. The irradiation time used was 20 minutes. After polymerization, the membrane was washed thoroughly with de-ionized water for 24hrs.

The mass gain of the resulting membrane after drying was 108.4 wt% and water flux was  $1048 \pm 4$  kg/m<sup>2</sup>h at 100 kPa giving Darcy permeability of  $6.47 \times 10^{-16}$ .

The protein (lysozyme) adsorption/desorption characteristics of the membrane were examined using the general procedure for a single membrane disk outlined earlier. A relatively

steep break through curve was obtained. The membrane had a lysozyme binding capacity of 88.7 mg/ml. The desorption experiment indicated that the recovery of protein was 64.0%.

#### Example 19

- 5 This example in combination with example 18 above further illustrates that the protein binding capacity and flow characteristics of a membrane can be tuned.

The monomer solution was produced by dilution of the solution formulated in Example 18 with acetone with the mass  
10 ratio of 1:1.

- A porous poly(propylene) support member in the form of a membrane, support PP1545-4, was used along with the general procedure for the preparation of composite materials described above. The irradiation time used was 90 minutes.  
15 After polymerization, the membrane was washed thoroughly with de-ionized water for 24hrs.

The mass gain of the resulting membrane after drying was 45.7 wt% and water flux was  $7319 \pm 180$  kg/m<sup>2</sup>h at 100 kPa.

- The protein (lysozyme) absorption/desorption characteristics  
20 of the membrane were examined using the general procedure for a single membrane disk outlined earlier. A relatively steep break through curve was observed. The membrane had a lysozyme binding capacity of 63.4 mg/ml. The desorption experiment indicated that the recovery of protein was 79.3%.

#### 25 Example 20

This example illustrates the effect of a neutral co-monomer on the protein binding capacity of composite materials of this invention.

Table 6: Chemical composition of stock solutions (amount of monomers in 10 mL of a solution)

Stock ID	AAM (g)	AMPS (g)	PEG2000XL (g)	BIS (g)	Irgacure® 2959, (g)	Solvents (Dioxane/DMF/H <sub>2</sub> O) volumetric ratio
S1	0.60	0.40	1.00	0.25	0.01	8:1:1
S2	0.40	0.60	1.00	0.25	0.01	8:1:1
S3	0.20	0.80	1.00	0.25	0.01	8:1:1
S4	0	1.00	1.00	0.25	0.01	8:1:1

PEG2000XL: PEG2000 diacrylate prepared in example 14

- 5 Monomer solutions were prepared by dilution of stock solutions S1-S4 in Table 6 with acetone with the mass ratio of 1:1.

- Composite membranes M1-M4 were prepared by using the corresponding diluted solutions of stocks S1-S4 and following the general preparation procedure described earlier. The porous support used was PP1545-4 and the irradiation time was 90 minutes. Upon completion of polymerization, the composite membranes were washed with de-ionized water for 24hrs.
- 15 The properties and protein binding capacities of composite membranes were examined and the results shown in Table 7. It

is evident that the charge density of polyelectrolyte gels influences significantly protein adsorption onto membranes.

Table 7: Properties and Lysozyme adsorption capacities of composite membranes

No.	Flux at 100kPa (kg/m <sup>2</sup> .h)	Binding Capacity (mg/ml)
M1	8146±96	56.9
M2	4273±46	76.3
M3	7940±303	41.5
M4	8651±72	16.4

## 5 Example 21

This example illustrates the effect of the chain length of the polyfunctional macromonomers used as cross-linkers (PEG diacrylates) on protein binding capacity of composite materials of this invention.

- 10 A series of stock solutions containing 0.6 g of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), 0.4 g of acrylamide (AAM), 0.10 g of *N,N'*-methylenebisacrylamide (BIS), 0.01 g of Irgacure® 2959, and 1.0 g of PEG diacrylate with different molecular weights(200, 1000, 2000, 4000),
- 15 obtained in Example 14, dissolved in 10 ml of a dioxane-DMF-water mixture with a volume ratio 80:10:10, respectively, was prepared. The stock solutions were subsequently diluted with acetone at the mass ratio of 1:1. A series of composite

membranes were prepared from these solutions using poly(propylene) support PP1545-4 and by following the general preparation procedure described above. The irradiation time used was set to 90 minutes. Upon  
 5 completion of polymerization, the composite membranes were washed with de-ionized water for 24hrs.

The properties and protein binding capacities of composite membranes were examined according to the general procedure for a single membrane disk. The results shown in Table 8  
 10 clearly indicate that the gel structure of composite membranes has substantial effect on protein adsorption. Possibly, it is related to the gel structure near the macropore surface, where an extremely loose structure may be formed that can allow protein to penetrate into the gel  
 15 layer at a certain depth. Another possibility is that by using a longer chain PEG diacrylate the surface area is increased owing to some fuzziness at the surface and thus making more adsorption site available to proteins.

Table 8: Properties and Lysozyme adsorption capacities of  
 20 composite membranes

PEG diacrylate	Flux at 100 kPa (kg/m <sup>2</sup> h)	Binding Capacity (mg/ml)
200	8390±218	24.4
1000	7275±139	58.1
2000	4273±46	76.3
4000	6039±111	75.4

## Example 22

This example illustrates the use of fibrous non-woven support to produce a composite material of this invention containing positively charged macroporous gel.

5 A 10 wt-% solution was prepared by dissolving diallyldimethylammonium chloride (DADMAC) and acrylamide (AAM), which were taken in the ratio 80:20, in a solvent mixture containing 65 wt-% of dioxane, 18 wt-% of water, and 17 wt-% of DMF. N,N'-methylenebisacrylamide (BIS) was added  
10 to the monomer solution to obtain 40% (mol/mol) cross-linking degree. The photoinitiator Irgacure® 2959 was added in the amount of 1% with respect to the total mass of the monomers.

A sample of the fibrous non-woven polypropylene substrate  
15 TR2611A was placed on a polyethylene sheet and filled with the monomer solution. The substrate was subsequently covered with another polyethylene sheet and the resulting sandwich was run between two rubber rollers to press the monomer solution into the pores and remove excess of  
20 solution. The filled substrate was irradiated at 350 nm for 20 min for the polymerization process to occur. The composite material was removed from between the polyethylene sheets, washed with water, TRIS-buffer solution and stored in water for 24 hrs. A duplicate sample was used to  
25 estimate the mass gain of the composite material. The substrate gained 45 % of the original weight in this treatment.

The composite material produced by this method had a water flux in the range of 2320 kg/m<sup>2</sup> hr at 70 kPa.

The protein (BSA) adsorption characteristic of a mono-layer of the composite material was examined using the general procedures one for a single membrane disk and one for a multi-membrane stack, as described above. The membrane stack  
5 contained 7 membrane layers of total thickness 1.75 mm. In both experiments the protein concentration was 0.4 g/L in a 50 mM TRIS buffer solution, and the flow rate of the protein solution used was  $3.1 \pm 0.1$  ml/min, delivered by peristaltic pump. The breakthrough capacity for BSA was 64 mg/ml in the  
10 single membrane experiment and  $55 \pm 2$  mg/ml in the multi-membrane stack experiment.

#### Example 23

This example illustrates the use of a mixture of two monomers in making a positively charged composite material  
15 of this invention.

A 10 wt-% solution was prepared by dissolving diallyldimethylammonium chloride (DADMAC) and (3-acrylamidopropyl)trimethylammonium chloride (APTAC), in the ratio 50 : 50, in a solvent mixture containing 65 wt-%  
20 dioxane, 18 wt-% water and 17 wt-% DMF. N,N'-methylenebisacrylamide (BIS) was added to the monomer solution to obtain 40% (mol/mol) cross-linking degree. The photoinitiator Irgacure<sup>®</sup> 2959 was added in the amount of 1% with respect to the total mass of the monomers.

25 A sample of the non-woven polypropylene substrate TR2611A was placed on a polyethylene sheet and filled with the monomer solution. The substrate was subsequently covered with another polyethylene sheet and the resulting sandwich was run between two rubber rollers to press the monomer  
30 solution into the pores and remove excess of solution. The filled substrate was irradiated at 350 nm for 20 min for the

polymerization process to occur. The composite material was removed from between the polyethylene sheets, washed with water, TRIS-buffer solution and stored in water for 24 hrs.

The composite material produced by this method had a water  
5 flux in the range of 2550 kg/m<sup>2</sup> hr at 70 kPa. The mass gain determined with a duplicate sample was found to be 45%.

The protein (BSA) adsorption characteristic of the mono-layer composite material was examined using the general procedure for a single membrane disk described above. A  
10 solution of BSA concentration of 0.4 g/L in a 50 mM TRIS buffer solution was delivered to the membrane at a flow rate of 2-4 ml/min. The breakthrough capacity of the composite material was 40 mg/ml.

#### Example 24

15 This example illustrates the effect of addition of a neutral monomer to the mixture of charged monomers used in example 23.

A 15 wt-% solution was prepared by dissolving diallyldimethylammonium chloride (DADMAC), (3-acrylamido-  
20 propyl)trimethylammonium chloride (APTAC), and acrylamide (AAM), which were taken in the ratio 40:40:20, in a solvent mixture containing of 65 wt-% dioxane, 17 wt-% of water, and 18 wt-% of DMF. N,N'-methylenebisacrylamide (BIS) was added to the monomer solution to obtain 20% (mol/mol) cross-  
25 linking degree. The photoinitiator Irgacure® 2959 was added in the amount of 1% with respect to the total mass of the monomers.

A sample of the non-woven polypropylene substrate TR2611A was placed on a polyethylene sheet and filled with the  
30 monomer solution. The substrate was subsequently covered



with another polyethylene sheet and the resulting sandwich was run between two rubber rollers to press the monomer solution into the pores and remove excess of solution. The filled substrate was irradiated at 350 nm for 20 min for the polymerization process to occur. The composite material was removed from between the polyethylene sheets, washed with water, TRIS-buffer solution and stored in water for 24 hrs.

The composite material produced by this method had a water flux of 550 kg/m<sup>2</sup> hr at 70 kPa and a mass gain (determined using a duplicate samples) of 65 wt-%.

The protein (BSA) adsorption characteristic of the monolayer composite material was examined using the general procedure for a single membrane disk described above. A solution of BSA concentration of 0.4 g/L in a 50 mM TRIS buffer solution was delivered to the membrane at a flow rate of 3.5-4 ml/min. The breakthrough capacity of the composite material was 130 mg/ml.

#### Example 25

This example illustrates the formation of unsupported positively charged macroporous gel by cross-linking of a preformed polymer.

A 10% solution of poly(allylamine hydrochloride) PAH was prepared by dissolving the polymer in a solvent mixture containing 60% water and 40% iso-propanol (2-propanol). The polymer was partially deprotonated (40%) by adding 6.67 N NaOH. Ethylene glycol diglycidyl ether (EDGE) was added to this solution to obtain 40% (mol/mol) degree of cross-linking. The solution was kept at room temperature for 3 hours for gel formation by the cross-linking reaction between the amine groups of PAH and epoxy groups of EDGE.

After 3 hours, the gel was placed in a water bath for all un-reacted chemicals to leach out.

A sample of the wet gel was examined using ESEM. The micrograph shown in Figure 14 indicates that a macroporous gel was formed with the pore diameter of about 70-80  $\mu\text{m}$ .  
The wet gel was mechanically very weak.

#### Example 26

This example illustrates the making macroporous gel incorporated in a non-woven fabric support.

10 A 10 wt-% solution of poly(allylamine hydrochloride) (PAH) was prepared as in Example 25. The polymer was partially deprotonated and EDGE added as described in Example 25 and the solution was applied to a sample of the non-woven polypropylene membrane support TR2611A placed between two  
15 polyethylene sheets. The resulting sandwich was run between two rubber rollers to press the polymer solution into the pores of the substrate, spread it evenly, and remove the excess solution. The solution-filled support sample was kept at room temperature for 3 hours for cross-linking  
20 process to take place resulting in the formation of gel. After that time, the composite material was removed from the sandwich and placed in a water bath for 12 hours to leach out unreacted chemicals.

A wet sample of the resulting composite membrane was  
25 examined using ESEM. The micrograph shown in Figure 15 indicates the composite membrane having macroporous gel in the fibrous non-woven support member. The average pore size of the gel was about 25-30  $\mu\text{m}$ . The membrane thickness was 800  $\mu\text{m}$  and the water flux measured at 100 kPa was 592  $\text{kg}/\text{m}^2\text{h}$ .

The composite material showed rather low BSA binding capacity of about 10 mg/ml.

#### Example 27

This example provides a comparison of the protein adsorption  
5 by a composite membrane of this invention with the commercial Mustang® Coin Q produced by Pall Corporation.

A composite material prepared in example 22 was tested in a multi-membrane stack of 7 membrane layers of a total thickness of 1.75 mm, according to the testing protocol  
10 described in example 22. A Mustang® Coin Q was also tested under similar conditions. The membrane stack was prepared by placing seven (7) layers of the membrane sample in the wet state on top of each other. The assembled membrane stack was lightly compressed pressed to remove excess of water.  
15 The membrane stack was then heated in an oven at 60-70°C for at least 30 min. The thickness of the resulting membrane stack in the dry state was 1.8-1.9 mm. This process produced a stack in which the multiple membrane layers adhered to each other. The results shown in Fig. 16 indicate that both  
20 systems give similar performances.

#### Example 28

This example provides the hydrodynamic (Darcy) permeability of reference composite materials containing porous support member and homogeneous gels filling the pores of the  
25 support. The homogeneous gels were obtained by using thermodynamically good solvents and their homogeneity was assessed based on transparency of simultaneously formed unsupported gels of the same composition. Clear and transparent gels were assumed to be homogeneous, contrary to  
30 macroporous gels that were always found opaque.

(A) Glycidyl methacrylate based homogeneous gel-filled composites

The composite materials containing homogeneous gels of glycidyl methacrylate-co-ethylene glycol dimethacrylate, GMA-co-EDMA, were prepared using 1,4-dioxane as a solvent and 4.7 wt-% of EDMA (cross-linker) in monomer mixture, and different total monomer concentrations. Poly(propylene) support PP1545-4 and the general procedure for preparing the composite materials of this invention were used. DMPA was used as photoinitiator and the irradiation was carried out at 350 nm for 120 minutes. The hydrodynamic permeability of the membranes was measured and an empirical equation was derived for the relationship between the hydrodynamic permeability,  $k$ , and the mass gain of the composite membranes containing poly(GMA-co-EDMA) homogeneous gels in the PP1545-4 support. The equation is as follows:

$$k = 3.62 \times 10^3 \times G^{-9.09}$$

The differences between the measured values of permeability and that calculated from the above equation were found to be less than  $\pm 3\%$ . This empirical relationship was subsequently used to calculate permeability of reference composite materials at different mass gains.

(B) Poly(diallyldimethylammonium chloride) based homogeneous gel-filled composites

The composite materials containing homogeneous gels of diallyldimethylammonium chloride-co-methylenebisacrylamide, DADMAC-co-BIS, were prepared using water as a solvent and 1.0 wt-% of BIS cross-linker in monomer mixture, and different total monomer concentrations. Poly(propylene) support PP1545-4 was coated with Triton X-114 surfactant by

immersing the support samples in 2t-% solution of the surfactant in methanol/water (60/40) mixture for 16 hours followed by drying in air. The general procedure for preparing the composite materials of this invention was used  
 5 to make homogeneous gel-filled membranes. Irgacure® 2959 was used as photoinitiator and the irradiation was carried out at 350 nm for 30-40 minutes. The hydrodynamic permeability of the series of membranes was measured and an empirical equation was derived for the relationship between  
 10 the Darcy permeability,  $k$ , and the mass gain,  $G$ :

$$k = 2.09 \times 10^{-12} \times G^{-4.01}$$

(C) Acrylamide based homogeneous gel-filled composites

The hydrodynamic permeability of homogeneous poly(acrylamide)-co-methylenebisacrylamide, AAM-co-BIS, was  
 15 estimated from the empirical relationship between the gel permeability and the gel polymer volume fraction provided by Kapur et al. in Ind. Eng. Chem. Res., vol. 35 (1996) pp. 3179-3185. According to this equation, the hydrodynamic permeability of a poly(acrylamide) gel,  
 20  $k_{gel} = 4.35 \times 10^{-22} \times \phi^{-3.34}$ , where  $\phi$  is the polymer volume fraction in the gel. In the same article, Kapur et al. provide a relationship between the hydrodynamic permeability of a gel and a porous membrane filled with the same gel. According to this relationship, the permeability of the membrane,  
 25  $k_{membrane} = (\epsilon/\tau) \times k_{gel}$ , where  $\epsilon$  is the porosity of the support and  $\tau$  is the tortuosity of the support pores. The pore tortuosity can be estimated as a ratio of the Kozeny constant,  $K$ , for a given porosity, i.e.,  $K = 5$ , and the Kozeny constant for a straight cylindrical capillary equal  
 30 to 2. Thus, for the poly(propylene) support PP1545-4 with porosity of 0.85, the ratio  $(\epsilon/\tau) = 0.85/2.5 = 0.34$ .

The polymer volume fraction,  $\phi$ , can be converted to mass gain using the partial specific volume,  $v_2$ , for

poly(acrylamide) and the density,  $\rho$ , of poly(propylene).

The values of these parameters can be found in Polymer

5 Handbook, edited by Brandrup et al., Chapter VII, Wiley and Sons, New York, 1999. Thus, the mass gain of a composite material containing poly(propylene) support of porosity  $\varepsilon$  filled with a gel whose polymer occupies the fraction  $\phi$  of the pores is given by

$$10 \quad \text{Mass Gain (\%)} = \frac{\phi/v_2}{(1-\varepsilon)\rho} \times 100\%$$

The above equation was combined with that of Kapur et al. to give an empirical relationship allowing one to calculate hydrodynamic permeability of reference composite materials,  $k$ , at different mass gains,  $G$ . The combined equation is as

15 follows:

$$k = 1.80 \times 10^{-12} \times G^{-3.34}$$

The equation is valid for  $\rho = 0.91 \text{ g/cm}^3$ ;  $\varepsilon = 0.85$ ;

$v_2 = 0.7 \text{ cm}^3/\text{g}$ ;  $(\varepsilon/\tau) = 0.34$ .

(D) Poly(AMPS) based homogeneous gel-filled composites

20 The composite materials containing homogeneous gels of 2-acrylamido-2-propane-1-sulfonic acid-co-methylenebisacrylamide, AMPS-co-BIS, were prepared using water as a solvent and 10.0 wt-% of BIS cross-linker in monomer mixture, and different total monomer concentrations.

25 Poly(propylene) support PP1545-4 was coated with Triton X-114 surfactant by immersing the support samples in 2t-% solution of the surfactant in methanol/water (60/40) mixture for 16 hours followed by drying in air. The general

procedure for preparing the composite materials of this invention was used to make homogeneous gel-filled membranes. Irgacure® 2959 was used as photoinitiator and the irradiation was carried out at 350 nm for 60 minutes. The hydrodynamic permeability of the series of membranes was measured and an empirical equation was derived for the relationship between the Darcy permeability,  $k$ , and the mass gain,  $G$ :

$$k = 2.23 \times 10^{-16} \times G^{-1.38}$$

10 (E) Poly(APTAC) based homogeneous gel-filled composites

The composite materials containing homogeneous gels of (3-acrylamidopropane)trimethylammonium chloride-co-methylenebisacrylamide, APTAC-co-BIS, were prepared using water as a solvent and 10.0 wt-% of BIS cross-linker in monomer mixture, and different total monomer concentrations. Poly(propylene) support PP1545-4 was coated with Triton X-114 surfactant by immersing the support samples in 2t-% solution of the surfactant in methanol/water (60/40) mixture for 16 hours followed by drying in air. The general procedure for preparing the composite materials of this invention was used to make homogeneous gel-filled membranes. Irgacure® 2959 was used as photoinitiator and the irradiation was carried out at 350 nm for 60 minutes. The hydrodynamic permeability of the series of membranes was measured and an empirical equation was derived for the relationship between the Darcy permeability,  $k$ , and the mass gain,  $G$ :

$$k = 9.51 \times 10^{-16} \times G^{-1.73}$$

(F) Poly(ethyleneimine) based homogeneous gel-filled composites

The composite materials containing homogeneous gels of branched poly(ethyleneimine) cross-linked with ethylene glycol diglycidyl ether (EDGE) were prepared using methanol solutions of BPEI of different concentrations. The degree of cross-linking used was 10 mol-%. Poly(propylene) support PP1545-4 was used together with the general procedure of fabrication of composite materials by in situ cross-linking of cross-linkable polymers described in Example 10. A series of membranes with different mass gains was prepared by changing the concentration of PEI in the solution. The Darcy permeability of the membranes was measured and an empirical equation describing the relationship between the permeability,  $k$ , and the mass gain,  $G$ , was derived. The equation is as follows:

$$k = 4.38 \times 10^{-14} \times G^{-2.49}$$

#### Example 29

This example provides comparison between Darcy permeability of composite materials of this invention containing supported macroporous gels and the permeability of the reference composite materials containing homogeneous gels filling the porous support member used in this invention.

The comparison is shown in Table 9 below.



Table 9: Permeability Ratio Values for Composite Materials

Composite Materials containing Macroporous Gels of this Invention			Darcy Permeability of Reference Composite Material	Permeability Ratio  $k_{\text{macroporous}}/k_{\text{homogeneous}}$
Example No.	Mass Gain %	Darcy Permeability  $k_{\text{macroporous}}$  $\text{m}^2$	$k_{\text{homogeneous}}$  $\text{m}^2$	
2	107	$9.53 \times 10^{-16}$	$2.93 \times 10^{-19}$	$3.2 \times 10^3$
5	74	$1.58 \times 10^{-15}$	$2.49 \times 10^{-18}$	$6.3 \times 10^2$
6	92*	$1.98 \times 10^{-18}$ *	$1.85 \times 10^{-18}$ *	$1.1 \times 10^0$ *
	100	$3.53 \times 10^{-16}$	$1.65 \times 10^{-18}$	$2.2 \times 10^2$
	100*	$5.19 \times 10^{-18}$ *	$1.65 \times 10^{-18}$ *	$3.2 \times 10^0$ *
7	103	$4.4 \times 10^{-15}$	$1.58 \times 10^{-18}$	$2.8 \times 10^3$
8	42	$9.87 \times 10^{-16}$	$8.81 \times 10^{-19}$	$1.1 \times 10^3$
9	80	$1.09 \times 10^{-16}$	$6.78 \times 10^{-20}$	$1.6 \times 10^3$
10	95	$1.89 \times 10^{-15}$	$5.40 \times 10^{-19}$	$3.5 \times 10^3$
11	144	$2.77 \times 10^{-15}$	$8.39 \times 10^{-19}$	$3.3 \times 10^1$
	158	$9.15 \times 10^{-16}$	$3.77 \times 10^{-17}$	$2.4 \times 10^1$
	237	$1.66 \times 10^{-17}$	$9.20 \times 10^{-19}$	$1.8 \times 10^1$
	265	$2.48 \times 10^{-15}$	$3.34 \times 10^{-19}$	$7.5 \times 10^3$
	277	$6.96 \times 10^{-16}$	$2.24 \times 10^{-19}$	$3.1 \times 10^3$

\* denotes homogeneous or micro-heterogeneous gels in composite materials (Comparative)

Table 9 (Continued)

Composite Materials containing Macroporous Gels of this Invention			Darcy Permeability of Reference Composite Material	Permeability Ratio $k_{\text{macroporous}}/k_{\text{homogeneous}}$
Example No.	Mass Gain %	Darcy Permeability $k_{\text{macroporous}}$ $\text{m}^2$	$k_{\text{homogeneous}}$ $\text{m}^2$	
12	103	$1.59 \times 10^{-18} *$	$3.38 \times 10^{-19} *$	$4.7 \times 10^0 *$
	108	$2.52 \times 10^{-18} *$	$2.93 \times 10^{-19} *$	$8.6 \times 10^0 *$
	110	$2.11 \times 10^{-18} *$	$2.70 \times 10^{-19} *$	$7.8 \times 10^0 *$
	112	$9.32 \times 10^{-18}$	$2.61 \times 10^{-19}$	$3.6 \times 10^1$
	130	$5.34 \times 10^{-16}$	$1.56 \times 10^{-19}$	$3.4 \times 10^3$
	273	$8.57 \times 10^{-17}$	$1.31 \times 10^{-20}$	$6.5 \times 10^3$
	307	$3.88 \times 10^{-17}$	$8.87 \times 10^{-21}$	$4.4 \times 10^3$
15	113	$2.26 \times 10^{-16}$	$1.39 \times 10^{-18}$	$1.6 \times 10^2$
16	51	$3.73 \times 10^{-15}$	$4.16 \times 10^{-18}$	$9.0 \times 10^2$
17	34	$7.52 \times 10^{-15}$	$7.18 \times 10^{-18}$	$1.0 \times 10^3$
18	108	$6.47 \times 10^{-16}$	$1.47 \times 10^{-18}$	$4.4 \times 10^2$
19	46	$4.52 \times 10^{-15}$	$4.85 \times 10^{-18}$	$9.3 \times 10^2$

\* denotes homogeneous or micro-heterogeneous gels in composite materials.

## Examples 30-37

These examples illustrate a method of preparing a responsive composite material of the present invention using photoinitiated free radical polymerization of acrylic acid

5 (AA) (ionic monomer), acrylamide (AA), and trimethylolpropane triacrylate (TRIM) as a cross-linker. The molar ratio of acrylic acid to acrylamide was 1:1 and 1,4-dioxane was used as a solvent in all experiments. Monomer solution compositions and polymerization conditions  
10 are given in Table 10. After polymerization, the responsive composite material was washed with de-ionized water for about 16 hrs.

TABLE 10: Monomer solution compositions and polymerization conditions

Example no.	Sample ID	Support Member	Total Concentration of Monomer Mixture (wt-%)	Degree of Cross-linking (mol-%)	Irradiation Time (min)	Mass Gain (%)
30	AM675	TR2611A	19.9	5.0	20	115.6
31	AM678	TR2611A	13.3	5.0	90	81.7
32	AM680	TR2611A	12.8	5.2	20	82.3
33	AM681	TR2611A	12.6	10.8	15	88.8
34	AM682	TR2611A	23.8	10.9	15	167.3
35	AM684	TR2611A	31.0	10.8	10	217.3
36	AM683	TR2611A	38.8	10.8	15	294.6
37	AM694	PP 1545-4	24.0	10.2	10	218.0

The amount of gel formed in the support member depends on the pore volume available to fill, the total concentration of the monomer mixture, and the degree of conversion in the polymerization. In Figure 17, the mass gain obtained with support TR2611A is plotted as a function of total monomer concentration. The data can be approximated to fall within a straight line ( $R^2 = 0.97$ ), indicating a similar degree of conversion for each sample. The experimental values are very close to their theoretical counterparts estimated from the pore volume in the support and the monomer concentration. This suggests that the degree of conversion is close to 100% and that an irradiation time of 10 minutes is sufficient under the light conditions applied. As expected, the mass gain obtained with the PP 1545-4 support was higher than that obtained with the TR2611A support due to the larger porosity of the former (85 vol-% versus 79.5 vol-%).

#### Example 38

This example illustrates the responsiveness of the responsive composite materials according to Example 30 to ionic interactions. For this purpose, the composite materials were tested with solutions of different pH and/or salt concentrations by measuring the flux at 100 kPa. A typical change in flux taking place with the change of pH from about 3 (1 mM HCl) to about 12 (1 mM NaOH), obtained with membrane AM675 is shown in Figure 18. It can be seen from the Figure that the flux measured with 1 mM HCl is nearly 100 times larger than the flux measured with 1 mM NaOH. The reason for this behavior of the membranes lies in changes in the degree of ionization of the acid component of the macroporous gel. At high pH (1 mM NaOH) the carboxyl groups of the acid component become ionized and the electrostatic repulsive force causes the polymer chains to

uncoil and stretch until balanced by counteracting forces of the polymer network elasticity and confinement imposed by the support member of the membrane. The swelling polymer chains reduce the pore volume and the pore radius in the gel. At low pH (1 mM HCl), the carboxyl groups are converted into neutral carboxylic acid groups, the electrostatic forces disappear, and the gel shrinks (collapses) enlarging the pores in the gel. The presence of the support member prevents the gel from collapsing as a whole, i.e., from the process that would occur in the unsupported bulk gel, and closing the pores. Thus, the presence of the support reverses the direction in which hydraulic properties of the gel change. When pure water flux is measured, the values obtained depend on the distance from equilibrium ionization at the water pH (~5.5). The initial water flux can be assumed to be measured at equilibrium. Immediately after the acid or base, the gel is far from equilibrium with water and the pure water flux reflects this state by being close to the flux in the ionized form (after NaOH) or neutral form (after HCl).

The ratio of the flux measured with 1 mM HCl to that measured with 1 mM NaOH has been taken as a measure of membrane response (MR). The results obtained with membranes described in Example 30-37 are shown in Table 11.

TABLE 11: Results for Examples 30-37

Membrane ID	AM675	AM678	AM680	AM681	AM682	AM684	AM683	AM694
Total Monomer Conc.	19.9	13.3	12.8	12.6	23.8	31.0	38.8	24.0
Wt-%								
Degree of Cross-linking	5.0	5.0	5.2	10.8	10.9	10.8	10.8	10.2
Mol-%								
Membrane Response (MR)	89.6	372.2	352.0	29.4	10.3	5.6	4.8	19.5

The results in Table 11 show that the response of the composite membranes of this invention to ionic interaction can also be controlled by the total concentration of monomer mixture and the degree of cross-linking. As the monomer concentration increases, the membrane sensitivity to the environmental changes decreases. Similar effect is found when the degree of cross-linking is increased.

#### 10 Example 39

This example illustrates the ability of membranes based on responsive composite materials of this invention to fractionate proteins. The separation of therapeutic proteins Human Serum Albumin (HSA) and Human Immunoglobulin G (HIgG) was chosen as a case study. Human plasma is the starting material for the production of a number of therapeutic proteins, which are referred to as plasma proteins. The most abundant amongst these are HSA and HIgG,

both of which are manufactured in bulk quantities. These proteins are generally fractionated by precipitation based processes which give high product throughput but poor resolution in terms of separation. Membrane based processes  
5 such as ultrafiltrations have the potential for giving both high throughput and high resolution.

Two composite membranes of this invention containing responsive macroporous gel, duplicates of membrane AM695 (see Tables 10 and 11), were tested for their suitability in  
10 separation of these plasma proteins. In the experiments discussed here, the change in membrane pore size with change in salt concentration was utilized to effect protein-protein separation in the manner desirable, i.e. sequential release from the membrane module. Other environmental conditions  
15 such as pH could well be used to achieve a similar objective.

A binary carrier phase system was used in the ultrafiltration experiments. The starting carrier phase in all the experiments was one with a low salt concentration  
20 (typically 5-10 mM NaCl). In all the experiments the carried phase was switched to one with a high salt concentration (typically 1 M NaCl). The change in salt concentration within the membrane module could be tracked by observing the conductivity of the permeate stream. The change in  
25 transmembrane pressure gave an idea about the change in membrane hydraulic permeability with change in salt concentration. Figure 19 shows the changes of transmembrane pressure and conductivity as a function of the permeate salt concentration (Fig 19. A and B) and the changes of  
30 transmembrane pressure as a function of permeate conductivity (Fig. 19C). In this experiment the salt concentration was being increased continuously in a linear



fashion. The transmembrane pressure observed is related to the permeate salt concentration and reflects changes in pore-diameter.

Experiments were carried out using human serum albumin and human immunoglobulin mixtures. The ultrafiltration was started at a low salt concentration (i.e. 10 mM). At this condition, human serum albumin was transmitted while human immunoglobulin G was almost totally retained. The salt concentration was then increased and this increased the pore diameter (as evident from drop in pressure in constant permeate flux ultrafiltration). This in turn led to the transmission of human immunoglobulin G through the membrane. Hence by altering the environmental condition it was possible to sequentially transmit proteins having different sizes through the same membrane. If the initial mixture had also contained a protein significantly larger than human immunoglobulin, it would have been possible to fractionate the three proteins (i.e. human serum albumin, human immunoglobulin and the significantly bigger protein) by appropriately controlling the change in salt concentration. Two of the three fractions obtained here would be in the permeate while the third fraction would be in the retentate.

The results obtained with duplicates of membrane AM694 are shown in Figures 20, 21, and 22. Figure 20 shows the results obtained with HIgG ultrafiltration. As evident from the figure, very little, if any HIgG was transmitted at the low salt concentration. However, when the salt concentration was increased, the HIgG was released from the membrane module. The drop in TMP with increase in salt concentration was due to the increase in pore diameter.

The results presented in Figure 21 were obtained with HSA ultrafiltration. As evident from the figure HSA was freely transmitted through the membrane even at low salt concentration. When the salt concentration was increased,  
5 the transmission of HSA was found to increase a bit.

Figure 22 shows the results obtained with HSA/HIgG ultrafiltration. At low salt concentration, HSA alone was transmitted. Ultrafiltration was continued until HSA was nearly completely removed from the membrane module. The HIgG  
10 was then released by increasing the salt concentration.

#### Example 40

This example illustrates a method for making a positively charged composite material of the invention having a high protein binding capacity.

15 A 15 wt-% solution was prepared by dissolving (3-acrylamidopropyl)-trimethylammonium chloride (APTAC), N-(hydroxymethyl)acrylamide and N,N'-methylenebisacrylamide as cross-linker in a molar ratio of 1:0.32:0.1, respectively, in a solvent mixture containing 10 wt-% water,  
20 60 wt-% di(propylene glycol)methyl ether and 30 wt-% dimethylformamide (DMF). The photo-initiator Irgacure® 2959 was added in the amount of 1% with respect to the mass of the monomers.

A sample of the fibrous non-woven polypropylene substrate  
25 TR2611A was placed on a polyethylene sheet and filled with the monomer solution. The substrate was subsequently covered with another polyethylene sheet and the resulting sandwich was run between two rubber rollers to press the monomer solution into the pores and to remove any excess  
30 solution. The substrate was irradiated for 5 minutes at

350 nm. The composite material was then removed from between the polyethylene sheets, washed with water and TRIS-buffer solution and stored in water for 24 hrs.

Several samples were prepared according to the above  
5 process, and the samples were then dried and weighed. The average mass gain of the composite material was 55.7% of the original weight of the starting support member.

The protein (BSA) adsorption characteristic of a multi-membrane stack of the above composite material was examined  
10 using the general procedure for a mono-layer of the composite material, as described earlier. The membrane stack tested contained 4 membrane layers, giving a total thickness 1.05 mm. The protein solution used was a 25 mM TRIS buffer solution with a protein concentration of  
15 0.4 g/L, and the flow rate of the protein solution was 5.0 ml/min at 150 kPa. The breakthrough capacity for BSA was 281 mg/ml. In a subsequent desorption step, approximately 85% of the BSA was recovered.

All references mentioned herein are incorporated herein by  
20 reference to the same extent as if each reference were stated to be specifically incorporated herein by reference.

To those skilled in the art, it is to be understood that many changes, modifications and variations could be made without departing from the spirit and scope of the present  
25 invention as claimed hereinafter.

CLAIMS:

1. A composite material that comprises a support member that has a plurality of pores extending through the support member and, located in the pores of the support member, and filling the pores of the support member, a macroporous cross-linked gel.
2. A composite material according to claim 1 that has a permeability ratio of at least 10.
3. A composite material according to claim 1 that has a permeability ratio of at least 100.
4. A composite material according to claim 1 that has a permeability ratio of at least 1000.
5. A composite material according to any one of claims 1 to 4, wherein the macroporous gel is non-self supporting.
6. A composite material according to any one of claims 1 to 5, wherein the macroporous cross-linked gel has macropores of average size between approximately 10 and approximately 3000 nm and a volume porosity between 30 and 80 %.
7. A composite material according to any one of claims 1 to 6, wherein the macroporous cross-linked gel has macropores of average size between 25 and 1500 nm.
8. A composite material according to any one of claims 1 to 6, wherein the macroporous cross-linked gel has macropores of average size between 50 and 1000 nm.

9. A composite material according to any one of claims 1 to 6, wherein the macroporous cross-linked gel has macropores of average size of about 700 nm.

10. A composite material according to any one of  
5 claims 1 to 9 in the form of a membrane for use as a filter in size exclusion separation.

11. A composite material according to any one of claims 1 to 9 in the form of a membrane and wherein the macroporous cross-linked gel bears charged moieties.

10 12. A composite material according to any one of claims 1 to 9 in the form of a membrane and in which the macroporous cross-linked gel bears a ligand for attachment of biological molecules, biological ions, or both.

13. A composite material according to any one of  
15 claims 1 to 12, wherein the support member has a void volume that is not completely occupied by the macroporous gel, and the density of the macroporous gel is greater at or adjacent to a first major surface of the support member than the density at or adjacent to a second major surface of the  
20 support member.

14. A composite material according to any one of claims 1 to 12, wherein the support member has a void volume that is substantially completely occupied by the macroporous gel.

25 15. A composite material according to any one of claims 1 to 14, wherein the support member is made of polymeric material in the form of a membrane that has a thickness of from between about 10 $\mu$ m to about 500  $\mu$ m,

contains pores of average size between 0.1 to 25  $\mu\text{m}$ , and has a volume porosity between 40 and 90 %.

16. A composite material according to any one of claims 1 to 15, wherein the support member is made of an extended polyolefin formed by phase separation.

17. The composite material according to any one of claims 1 to 15, wherein the support member comprises a polymeric material selected from the group consisting of polysulfones, polyethersulfones, polyphenyleneoxides, polycarbonates, polyesters, cellulose and cellulose derivatives.

18. A composite material according to any one of claims 1 to 14, wherein the support member is made of polymeric material in the form of a fibrous woven or non-woven fabric that has a thickness of from between about 10  $\mu\text{m}$  to about 2000  $\mu\text{m}$ , contains pores of average size between 0.1 to 25  $\mu\text{m}$ , and has a volume porosity between 40 and 90 %.

19. A composite material according to any one of claims 1 to 18, wherein the support member comprises a stack of 2 to 10 separate support members.

20. A composite material according to any one of claims 1 to 19, wherein the macroporous cross-linked gel is a neutral or charged hydrogel, a polyelectrolyte gel, a hydrophobic gel, a neutral gel, or a functional gel.

21. A composite material according to claim 20, wherein the neutral or charged hydrogel is selected from the group consisting of cross-linked poly(vinyl alcohol), poly(acrylamide), poly(isopropylacrylamide), poly(vinylpyrrolidone), poly(hydroxymethyl acrylate),

poly(ethylene oxide), copolymers of acrylic acid or methacrylic acid with acrylamide, isopropylacrylamide, or vinylpyrrolidone, copolymers of acrylamide-2-methyl-1-propanesulfonic acid with acrylamide, isopropylacrylamide, or vinylpyrrolidone, copolymers of (3-acrylamidopropyl) trimethylammonium chloride with acrylamide, isopropylacrylamide, or N-vinylpyrrolidone, and copolymers of diallyldimethylammonium chloride with acrylamide, isopropylacrylamide, or vinylpyrrolidone.

22. A composite material according to claim 20, wherein the polyelectrolyte gel is selected from the group consisting of cross-linked poly(acrylamido-2-methyl-1-propanesulfonic acid) and its salts, poly(acrylic acid) and its salts, poly(methacrylic acid) and its salts, poly(styrenesulfonic acid) and its salts, poly(vinylsulfonic acid) and its salts, poly(alginic acid) and its salts, poly[(3-acrylamidopropyl)trimethylammonium] salts, poly(diallyldimethylammonium) salts, poly(4-vinyl-N-methylpyridinium) salts, poly(vinylbenzyl-N-trimethylammonium) salts, and poly(ethyleneimine) and its salts.

23. A composite material according to claim 20, wherein the hydrophobic gel is selected from the group consisting of cross-linked polymers or copolymers of ethyl acrylate, n-butyl acrylate, propyl acrylate, octyl acrylate, dodecyl acrylate, octadecylacrylamide, stearyl acrylate, and styrene.

24. A composite material according to claim 20, wherein the neutral gel is selected from the group consisting of cross-linked polymers or copolymers of acrylamide, N,N-dimethylacrylamide,

N-methacryloylacrylamide, N-methyl-N-vinylacetamide, and N-vinylpyrrolidone.

25. A composite material according to claim 20, wherein the functional gel has functional groups that take the form of antibodies, amino acid ligands, antigen and antibody ligands, dye ligands, and metal affinity ligands.

26. A composite material according to any one of claims 1 to 25, wherein the macroporous cross-linked gel comprises a macromonomer.

27. A composite material according to claim 26, wherein the macromonomer is selected from the group consisting of poly(ethylene glycol) acrylate and poly(ethylene glycol) methacrylate.

28. A composite material according to any one of claims 1 to 27, wherein the macroporous cross-linked gel is cross-linked by a polyfunctional macromonomer.

29. A composite material according to claim 28, wherein the polyfunctional macromonomer is selected from the group consisting of poly(ethylene glycol) diacrylate and poly(ethylene glycol) dimethacrylate.

30. A composite material according to any one of claims 1 to 19, wherein the macroporous cross-linked gel is a positively charged hydrogel comprising a co-polymer of (3-acrylamidopropyl)-trimethylammonium chloride (APTAC) and N-(hydroxymethyl)acrylamide cross-linked by N,N'-methylenebisacrylamide.

31. A composite material according to any one of claims 1 to 30, wherein the macroporous cross-linked gel is a responsive macroporous cross-linked gel.



32. A composite material according to claim 31, wherein the responsive macroporous cross-linked gel can be in:

- a) a stable collapsed state, wherein the average size of the  
5 macropores in the gel is from 10 to 2000 nm,
- b) a stable swollen state, wherein the average size of the macropores in the gel is from 2 to 200 nm, or
- c) one of multiple stable intermediate states, wherein the  
average size of the macropores in the gel is between 2 and  
10 2000 nm.

33. A composite material according to claim 31 or 32, wherein the responsive macroporous cross-linked gel is responsive to variations in at least one of pH, ionic strength, temperature, light intensity, or electrochemical  
15 current.

34. A composite material according to claim 31, 32, or 33, wherein the responsive macroporous cross-linked gel comprises a responsive monomer, a neutral monomer and a cross-linking agent.

20 35. A process for size-exclusion filtration which comprises passing a liquid to be filtered through a composite material according to any one of claims 1 to 34.

36. A process according to claim 35, wherein the liquid is a suspension of cells or a suspension of  
25 aggregates.

37. A process for Donnan exclusion separation which comprises passing a liquid containing a charged material through a composite material according to any one of

claims 1 to 34, which composite material bears charges on the macroporous gel.

38. A process for adsorbing a biological molecule or a biological ion from a liquid, which comprises passing a liquid containing the biological molecule or biological ion through a composite material according to claim 1, which composite material bears binding sites that display specific interactions for the biomolecule on the macroporous gel.

39. A process according to claim 38, wherein the biological molecule or the biological ion is selected from the group consisting of albumins, lysozyme, viruses, cells,  $\gamma$ -globulins of human and animal origins, immunoglobulins of both human and animal origins, proteins of recombinant or natural origin including, polypeptides of synthetic or natural origin, interleukin-2 and its receptor, enzymes, monoclonal antibodies, trypsin and its inhibitor, cytochrome C, myoglobin, recombinant human interleukin, recombinant fusion protein, nucleic acid derived products, DNA of either synthetic or natural origin, and RNA of either synthetic or natural origin.

40. A process according to claim 38 or 39, wherein the specific interactions are electrostatic interactions.

41. A process according to claim 38 or 39, wherein the specific interactions are affinity interactions.

42. A process according to claim 38 or 39, wherein the specific interactions are hydrophobic interactions.

43. A process for solid phase chemical synthesis which comprises passing a liquid, having a first reactant through a composite material according to any one of claims 1 to 34,

wherein a second reactant is in a macropore of the composite material.

44. A process for preparing a composite material that comprises a support member that has a plurality of pores  
5 extending through the support member and, located in the pores of the support member and filling the pores of the support member, a macroporous cross-linked gel, the process comprising:

a) introducing into the pores of the support member a  
10 solution or suspension of

i) one or more monomers and one or more cross-linking agents that can combine to form a macroporous gel,  
or

ii) one or more cross-linkable polymers and one or  
15 more cross-linking agents that can combine to form a macroporous gel,

b) reacting the monomers and the cross-linking agent or the polymer and the cross-linking agent to form a macroporous cross-linked gel that fills the pores of the support member.

20 45. A process according to claim 44, wherein the molar ratio of cross-linking agent to monomers is in the range of from about 5:95 to about 70:30.

46. A process according to claim 44, wherein the molar ratio of cross-linking agent to monomers is in the range of  
25 from about 10:90 to about 50:50.

47. A process according to claim 44, wherein the molar ratios of cross-linking agent to monomers is in the range of from about 15:85 to about 45:55.

48. A process according to any one of claims 44 to 47, wherein the monomer is selected from the group consisting of acrylamide, 2-acryloxyethyltrimethylammonium chloride, N-acryloxysuccinimide,

- 5 N-acryloyltris(hydroxymethyl)methylamine, 2-aminoethyl methacrylate hydrochloride, N-(3-aminopropyl)methacrylamide hydrochloride, butyl acrylate and methacrylate, N,N-diethylacrylamide, N,N-dimethylacrylamide, 2-(N,N-dimethylamino)ethyl acrylate and methacrylate,
- 10 N-[3-(N,N-dimethylamino)propyl]methacrylamide, N,N-dimethylacrylamide, n-dodecyl acrylate, n-dodecyl methacrylate, dodecyl methacrylamide, ethyl methacrylate, 2-(2-ethoxyethoxy)ethyl acrylate and methacrylate, 2,3-dihydroxypropyl acrylate and methacrylate, glycidyl
- 15 acrylate and methacrylate, n-heptyl acrylate and methacrylate, 1-hexadecyl acrylate and methacrylate, 2-hydroxyethyl acrylate and methacrylate, N-(2-hydroxypropyl)methacrylamide, hydroxypropyl acrylate and methacrylate, methacrylamide, methacrylic anhydride,
- 20 methacryloxyethyltrimethylammonium chloride, 2-(2-methoxy)ethyl acrylate and methacrylate, octadecyl acrylamide, octylacrylamide, octyl methacrylate, propyl acrylate and methacrylate, N-iso-propylacrylamide, stearyl acrylate, styrene, 4-vinylpyridine, vinylsulfonic acid,
- 25 N-vinyl-2-pyrrolidinone, particularly preferred monomers include dimethyldiallylammonium chloride, acrylamido-2-methyl-1-propanesulfonic acid (AMPS), (3-acrylamidopropyl) trimethylammonium chloride (APTAC), acrylamide, methacrylic acid (MAA), acrylic acid (AA), 4-styrenesulfonic acid and
- 30 its salts, acrylamide, glycidyl methacrylate, diallylamine, and diallylammonium chloride. and sodium styrenesulfonate.

49. A process according to any one of claims 44 to 47, wherein the monomer is selected from the group consisting of

dimethyldiallylammonium chloride, acrylamido-2-methyl-1-propanesulfonic acid (AMPS), (3-acrylamidopropyl) trimethylammonium chloride (APTAC), acrylamide, methacrylic acid (MAA), acrylic acid (AA), 4-styresulfonic acid and its salts, acrylamide, hydroxyalkylacrylamides, glycidyl methacrylate, diallylamine, and diallylammonium chloride.

50. A process according to any one of claims 44 to 47, wherein one or more monomers are macromonomers.

51. A process according to claim 50 or 51, wherein the macromonomers have a molecular weight greater than about 200 Da.

52. A process according to claim 50, 51 or 52, wherein the macromonomers are poly(ethylene glycol) acrylate or poly(ethylene glycol) methacrylate.

53. A process according to claim 50, wherein the cross-linking agent is selected from the group consisting of bisacrylamidoacetic acid, 2,2-bis[4-(2-acryloxyethoxy)phenyl]propane, 2,2-bis(4-methacryloxyphenyl)propane, butanediol diacrylate and dimethacrylate, 1,4-butanediol divinyl ether, 1,4-cyclohexanediol diacrylate and dimethacrylate, 1,10-dodecanediol diacrylate and dimethacrylate, 1,4-diacryloylpiperazine, diallylphthalate, 2,2-dimethylpropanediol diacrylate and dimethacrylate, dipentaerythritol pentaacrylate, dipropylene glycol diacrylate and dimethacrylate, N,N-dodecamethylenebisacrylamide, glycerol trimethacrylate, glycerol tris(acryloxypropyl) ether, N,N'-hexamethylenebisacrylamide, N,N'-octamethylenebisacrylamide, 1,5-pentanediol diacrylate and dimethacrylate, 1,3-phenylenediacylate, poly(ethylene

glycol) (n) diacrylate and dimethacrylate, poly(propylene) (n) diacrylate and dimethacrylate, triethylene glycol diacrylate and dimethacrylate, triethylene glycol divinyl ether, and tripropylene glycol diacrylate and dimethacrylate,  
5 trimethylolpropane triacrylate, and divinylbenzene.

54. A process according to any one of claims 44 to 49, wherein the cross-linking agent is selected from the group consisting of N,N'-methylenebisacrylamide, diethylene glycol diacrylate and dimethacrylate, ethylene glycol diacrylate  
10 and dimethacrylate, tetra(ethylene glycol) diacrylate, 1,6-hexanediol diacrylate, divinylbenzene, trimethylolpropane triacrylate, and poly(ethylene glycol) diacrylate.

55. A process according to any one of claims 44 to 49,  
15 wherein the cross-linking agent is a polyfunctional macromonomer.

56. A process according to claim 55, wherein the polyfunctional macromonomer has molecular weight greater than about 200 Da.

20 57. A process according to claim 56, wherein the polyfunctional macromonomer is poly(ethylene glycol) diacrylate or poly(ethylene glycol) dimethacrylate.

58. A process according to claim 44, wherein the cross-linkable polymer is selected from the group consisting  
25 of poly(ethyleneimine), poly(4-vinylpyridine), poly(vinylbenzyl chloride), poly(diallylammonium chloride), poly(glycidyl methacrylate), copolymers of vinylpyridine and dimethyldiallylammonium chloride, and copolymers of vinylpyridine, dimethyladiallylammonium chloride, or

(3-acrylamidopropyl)trimethylammonium chloride with glycidyl methacrylate.

59. A process according to claim 44, wherein the cross-linkable polymer is selected from the group consisting of poly(ethyleneimine), poly(diallylammonium chloride), and poly(glycidyl methacrylate).

60. A process according to claim 44, 58 or 59, wherein the cross-linking agent is selected from the group consisting of ethylene glycol diglycidyl ether, poly(propylene glycol) diglycidyl ether, 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibromohexane,  $\alpha,\alpha'$ -dibromo-p-xylene,  $\alpha,\alpha'$ -dichloro-p-xylene, 1,4-dibromo-2-butene, piperazine, 1,4-diazabicyclo[2.2.2]octane, 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, 1,7-diaminoheptane, 1,8-diaminooctane.

61. A process according to any one of claims 44 to 60, wherein a photoinitiator is added prior to macroporous cross-linked gel formation.

62. A process according to claim 61, wherein the photoinitiator is selected from the group consisting of 2-hydroxy-1[4-(2-hydroxyethoxy)phenyl]2-hydroxy-2-methyl-1-propane-1-one, 2,2-dimethoxy-2-phenylacetophenone (DMPA), benzophenone, benzoin and benzoin ethers, dialkoxyacetophenones, hydroxyalkylphenones, and  $\alpha$ -hydroxymethyl benzoin sulfonic esters.

63. A process according to any one of claims 44 to 60, wherein a thermoinitiator is added prior to macroporous cross-linked gel formation.

64. A process according to claim 63, wherein the thermoinitiator is selected from the group consisting of 1,1'-azobis(cyclohexanecarbonitrile), azobis(isobutyronitrile) (AIBN), potassium persulfate, ammonium persulfate, and benzoyl peroxide.

65. A process according to any one of claims 44 to 64, wherein a porogen is added prior to macroporous cross-linked gel formation.

66. A process according to claim 65 wherein the porogen is a poor solvent for the macroporous cross-linked gel.

67. A process according to any one of claims 44 to 66, wherein the formed macroporous cross-linked gel contains reactive functional groups selected from the group consisting of epoxy, anhydride, azide, reactive halogen, and acid chloride groups, to which functional groups are then reacted.

68. A process according to any one of claims 44 to 66, wherein the formed macroporous cross-linked gel contains groups that can be quaternized through a subsequent reaction to give a charged macroporous cross-linked gel.

69. A process for chromatographic filtration of a solution or suspension containing two or more species of different size that are dissolved or suspended in a fluid, which process comprises (a) passing the fluid through a composite material as claimed in claim 31 so that species of the smallest size pass through the composite material but larger species do not pass through the composite material, and (b) changing an environmental condition to increase the

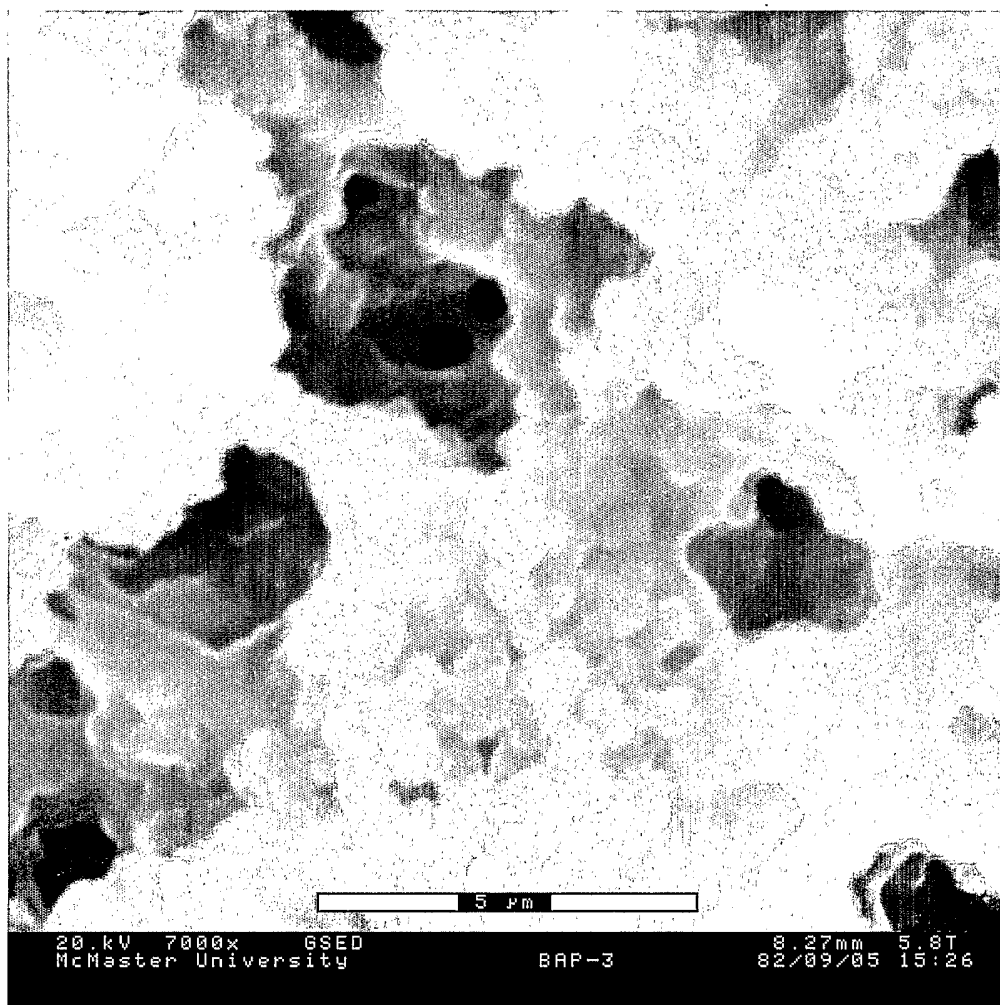


size of the pores in the macroporous gel so that species of a next larger size pass through the composite material.

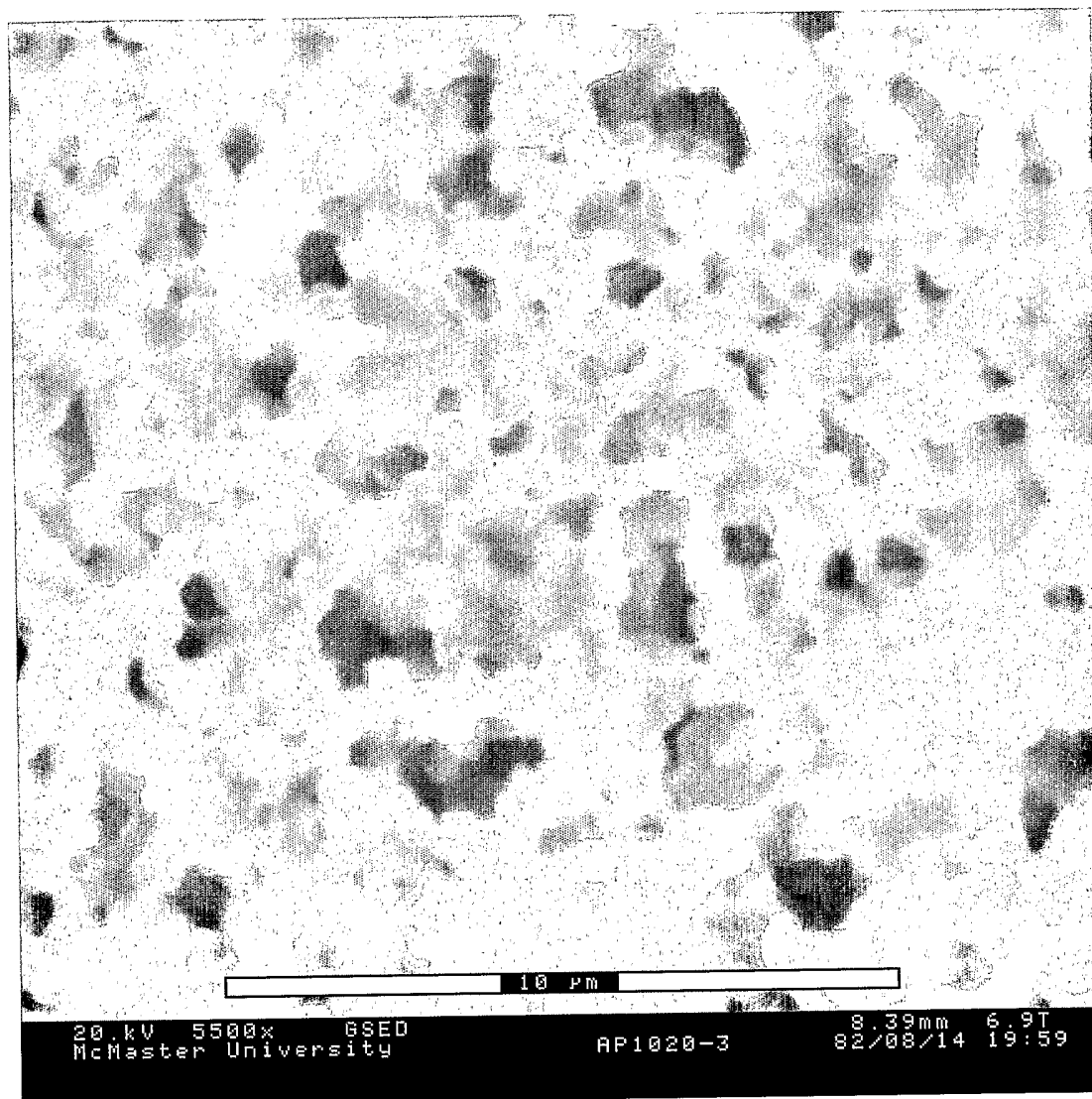
70. The process according to claim 69, wherein process step (b) is repeated to allow sequential elution of larger  
5 species.

71. The process according to claim 69 or 70, wherein the species are selected from proteins, cells, aggregates and particles.

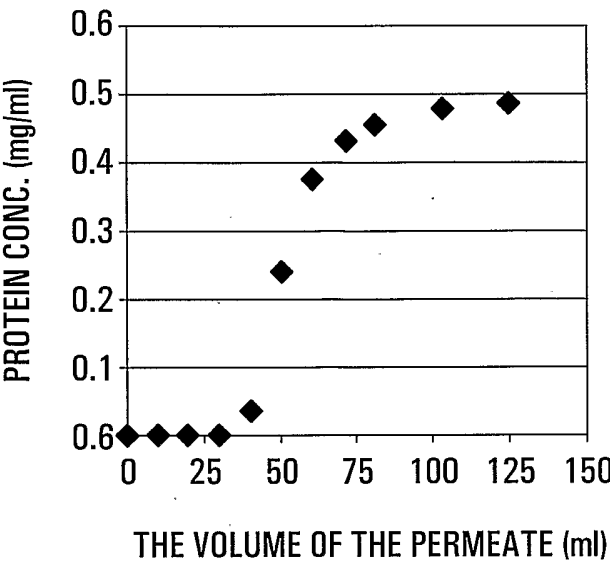
72. The process according to claim 69, 70 or 71,  
10 wherein the environmental condition is selected from the group consisting of pH, ionic strength, temperature, light intensity, and electrochemical current or a combination thereof.



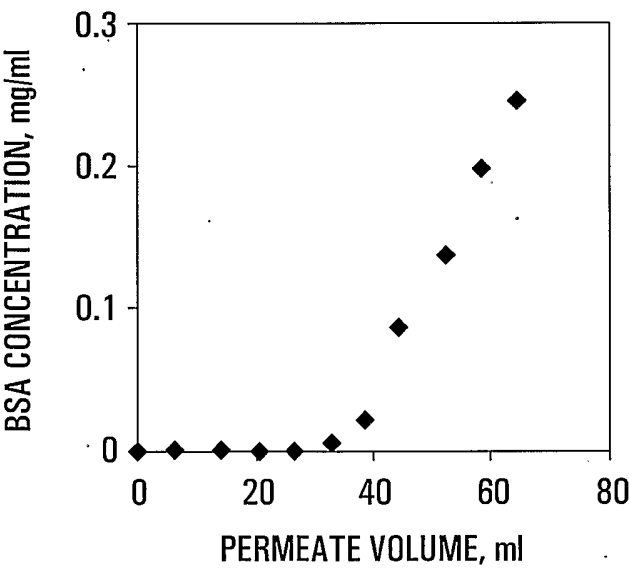
**FIG. 1**



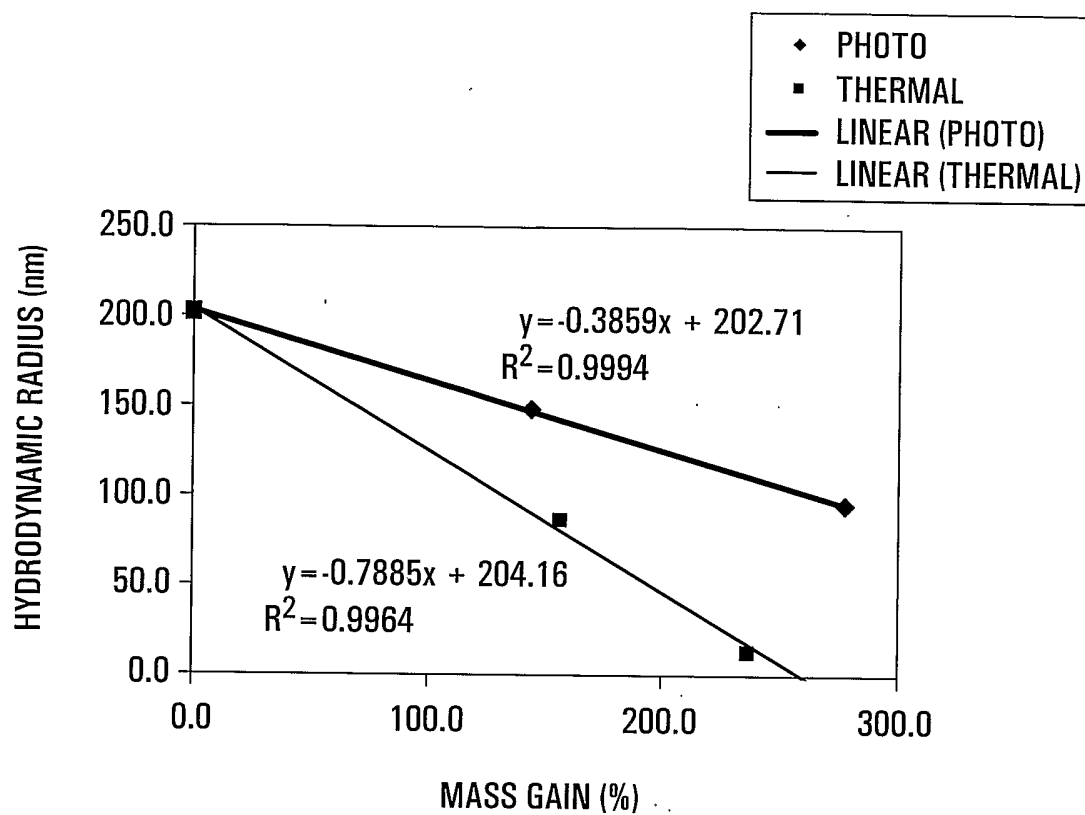
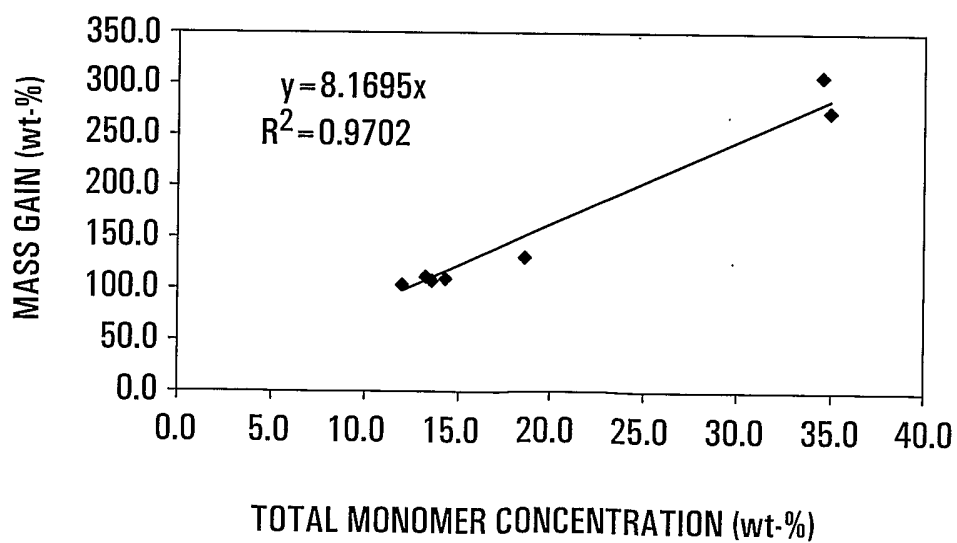
**FIG. 2**

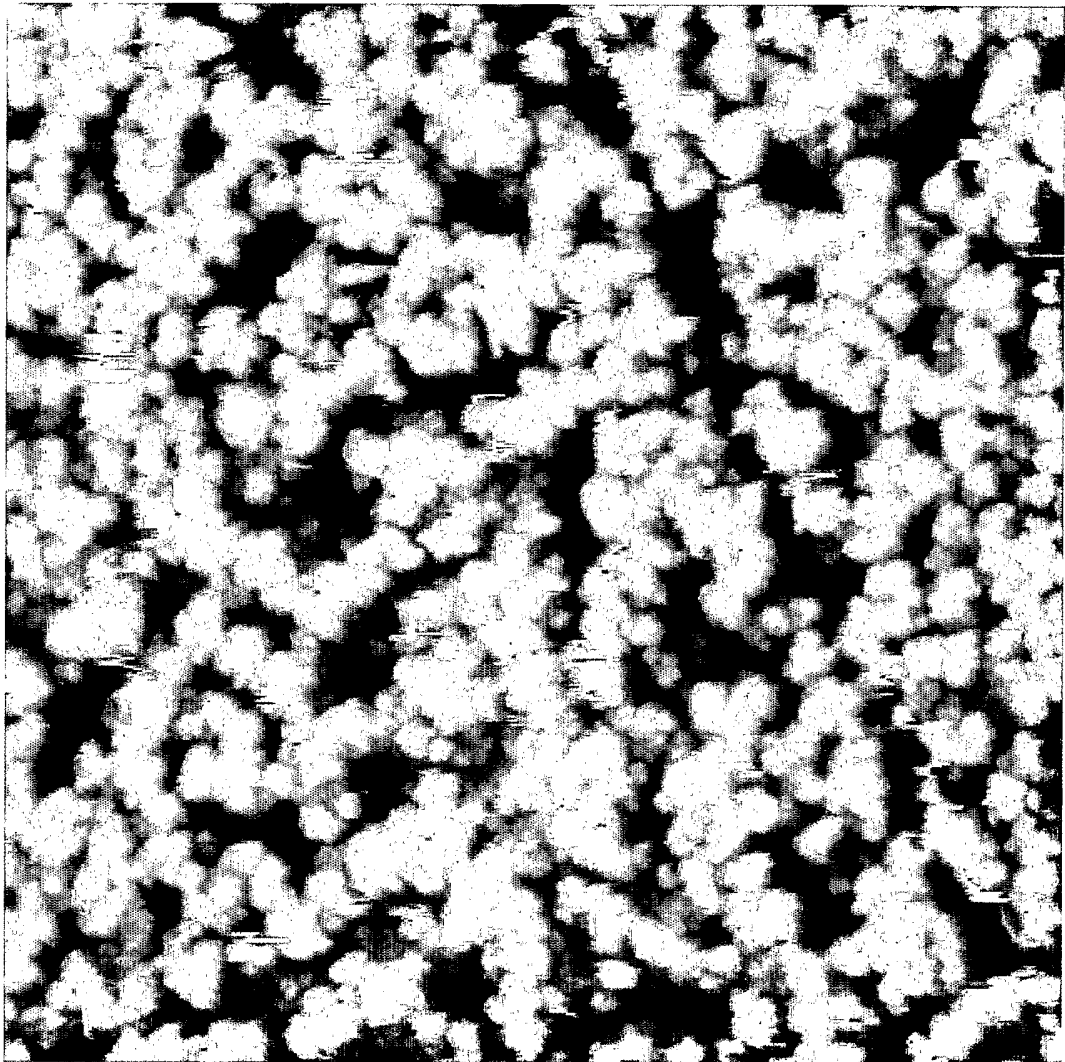


**FIG. 3**

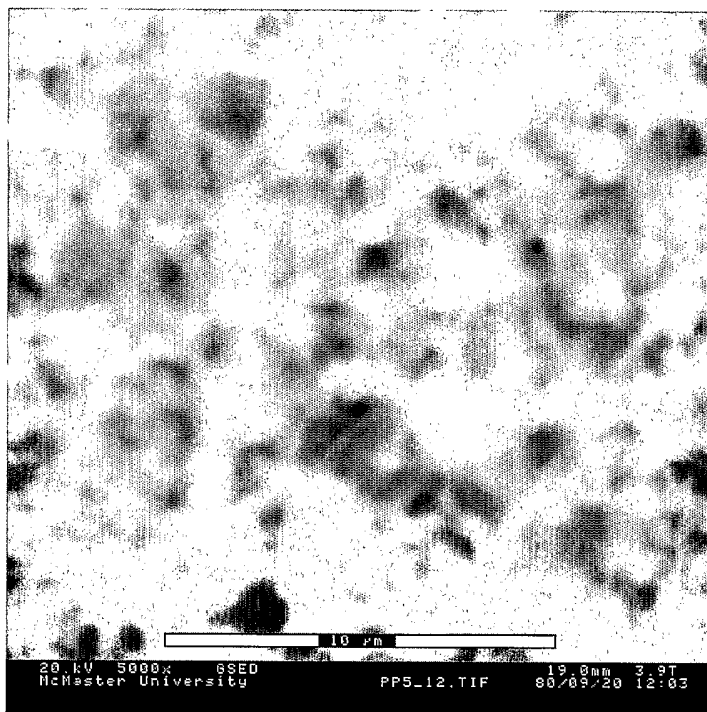


**FIG. 4**

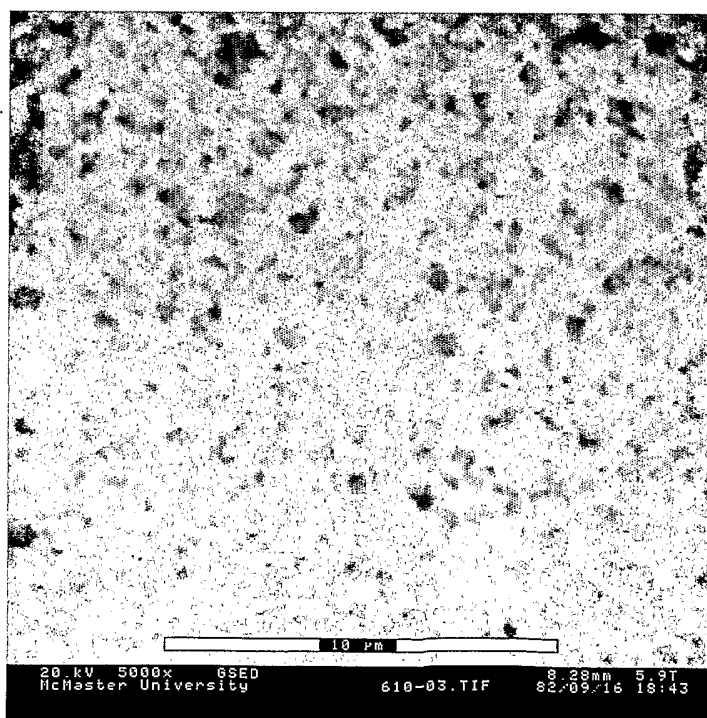
**FIG. 5****FIG. 6**



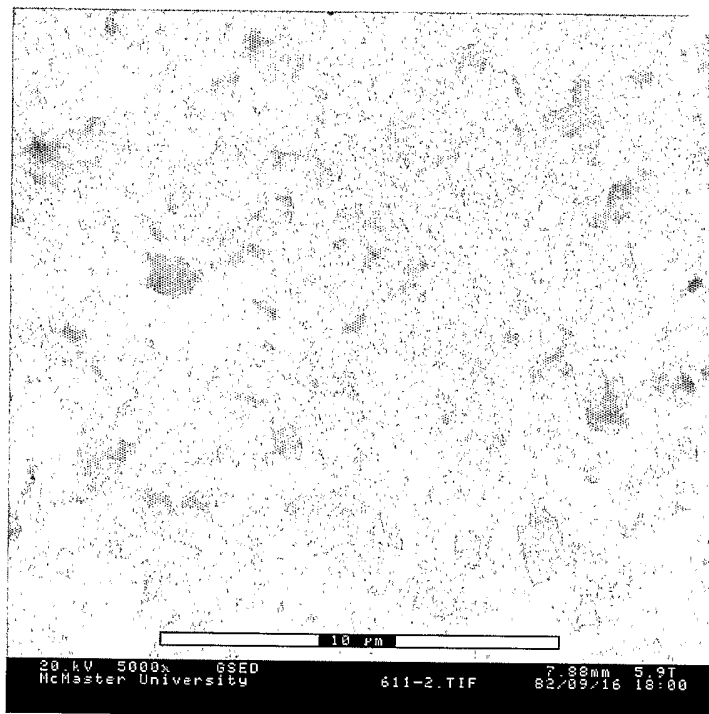
**FIG. 7**



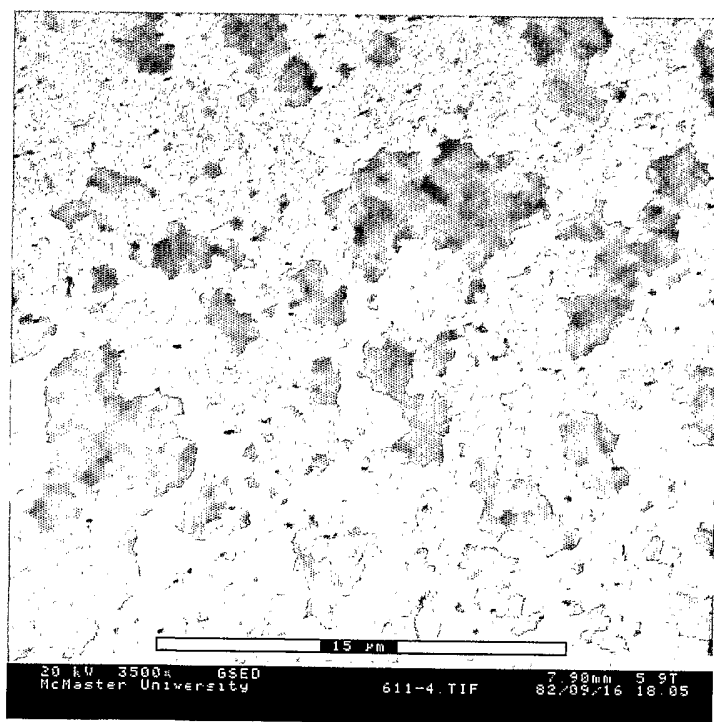
**FIG. 8A**



**FIG. 8B**

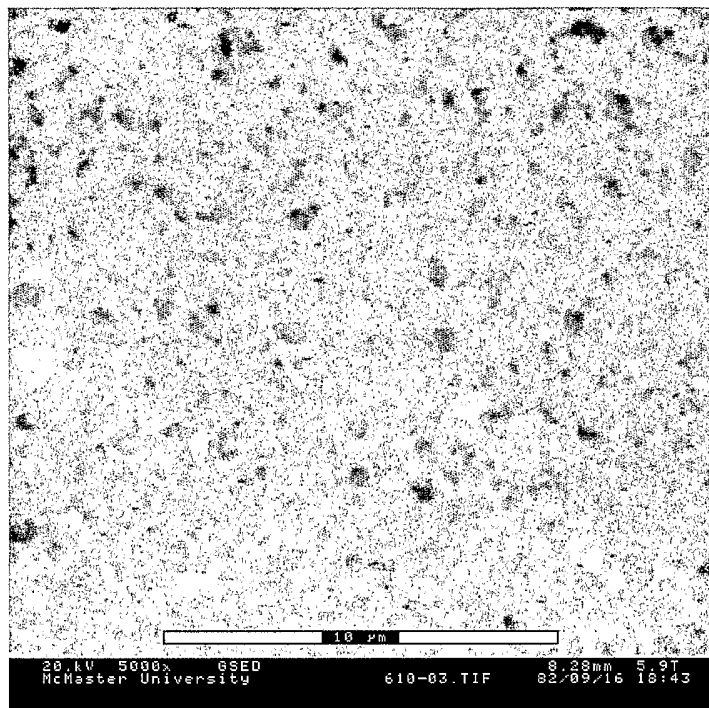


**FIG. 9A**

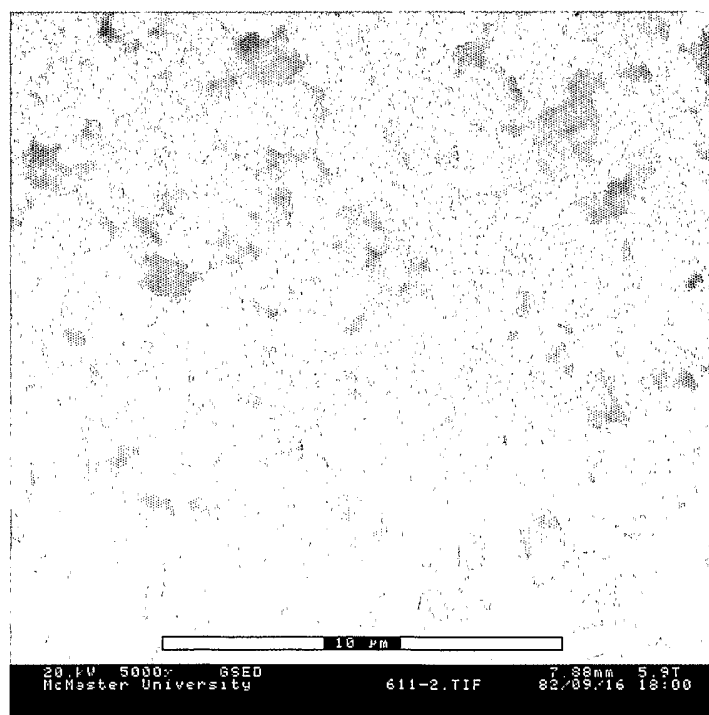


**FIG. 9B**

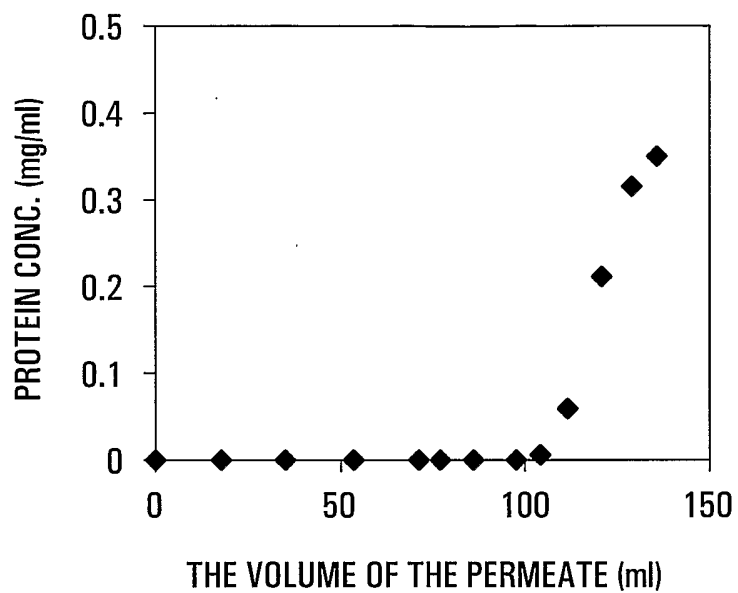
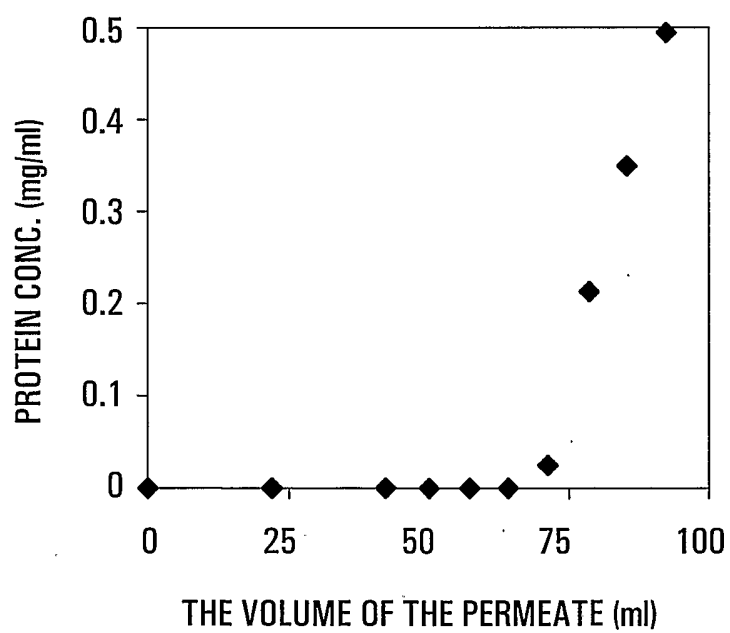


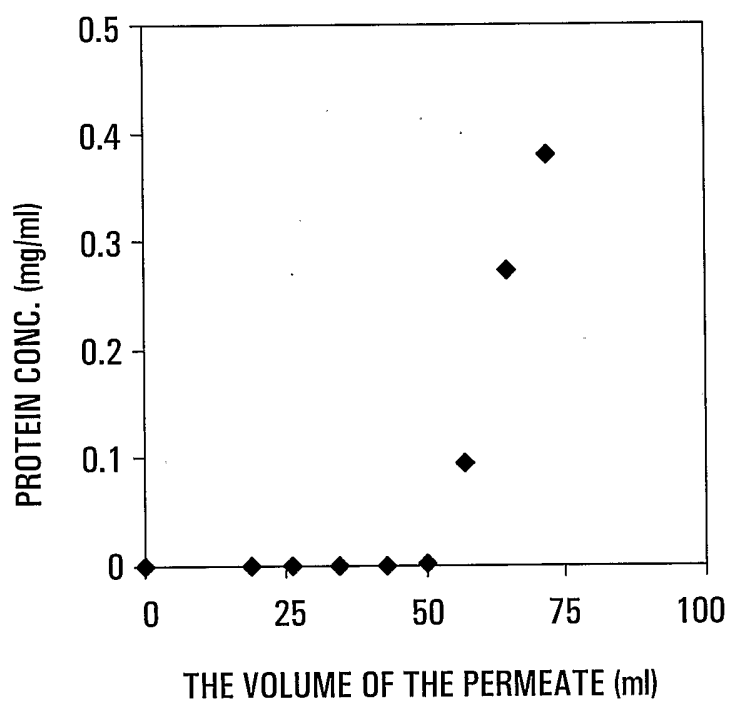


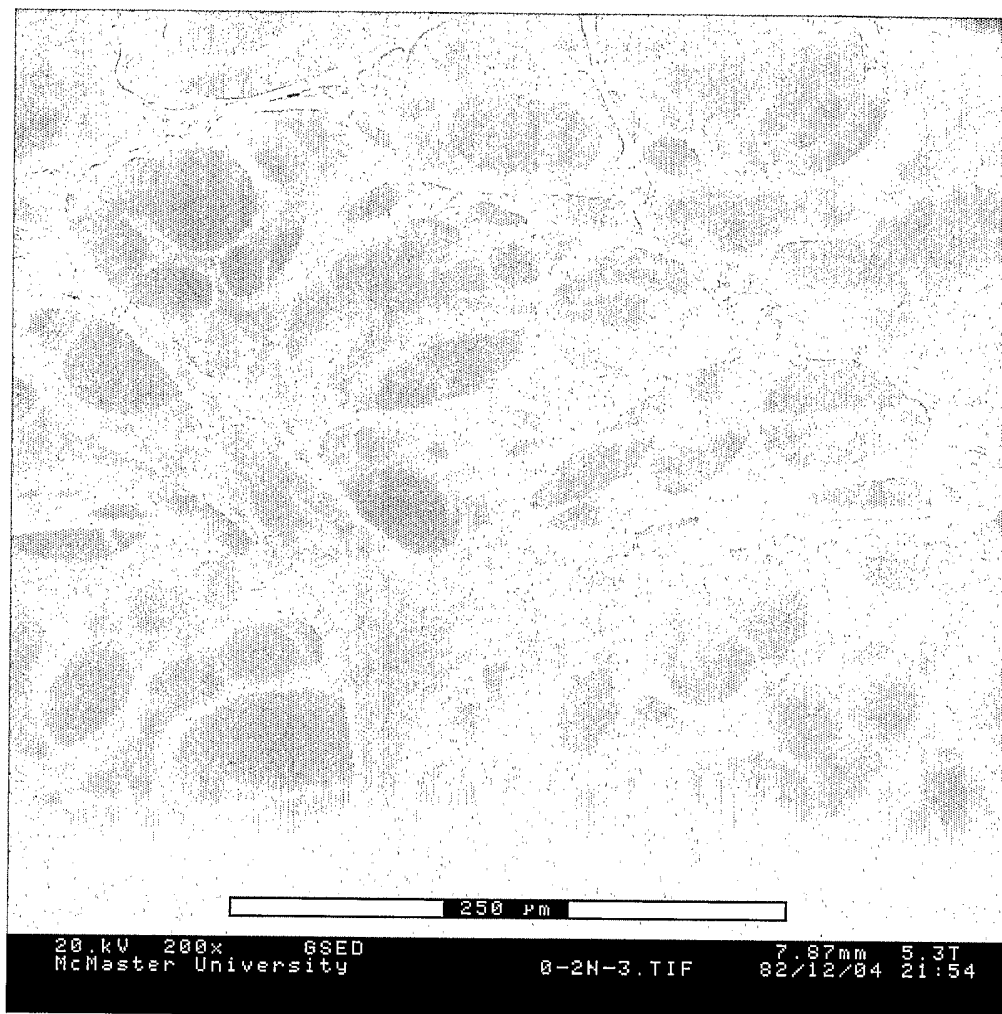
**FIG. 10A**



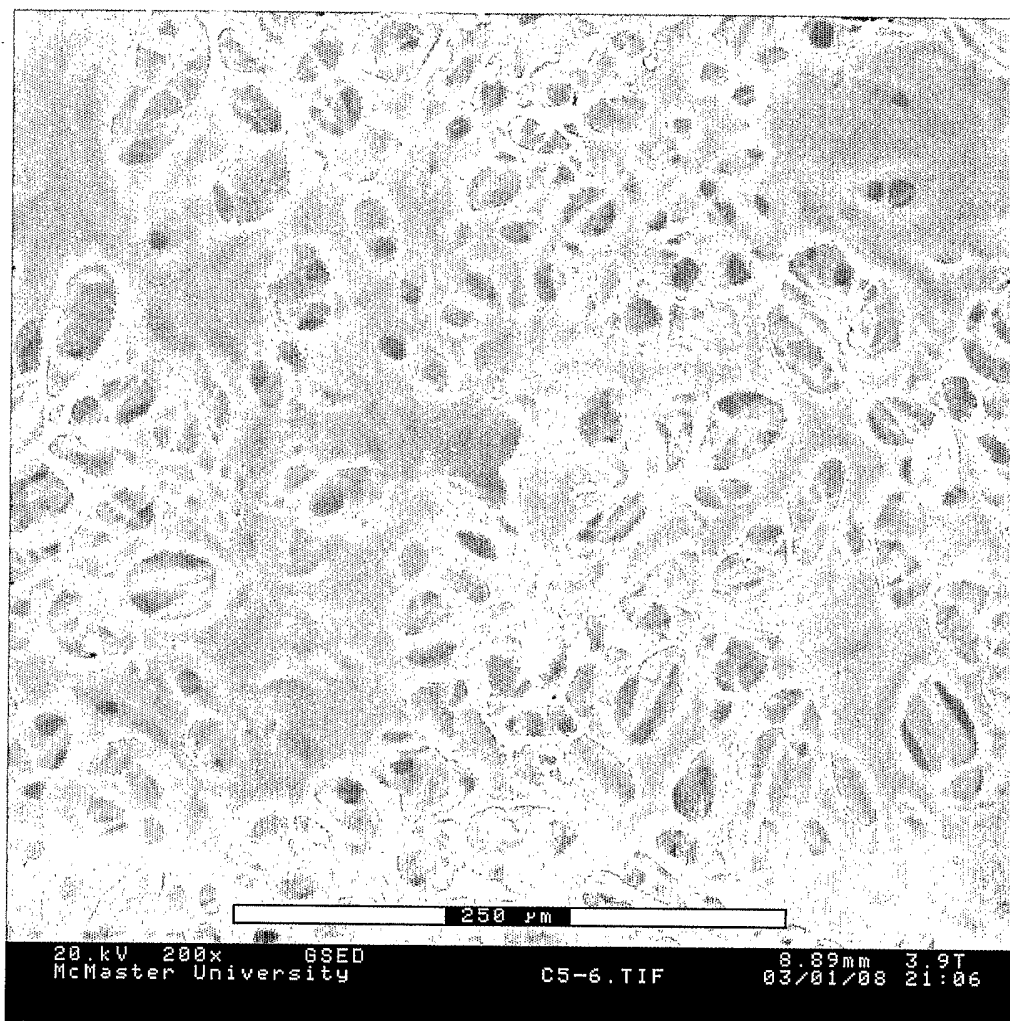
**FIG. 10B**

**FIG. 11****FIG. 12**

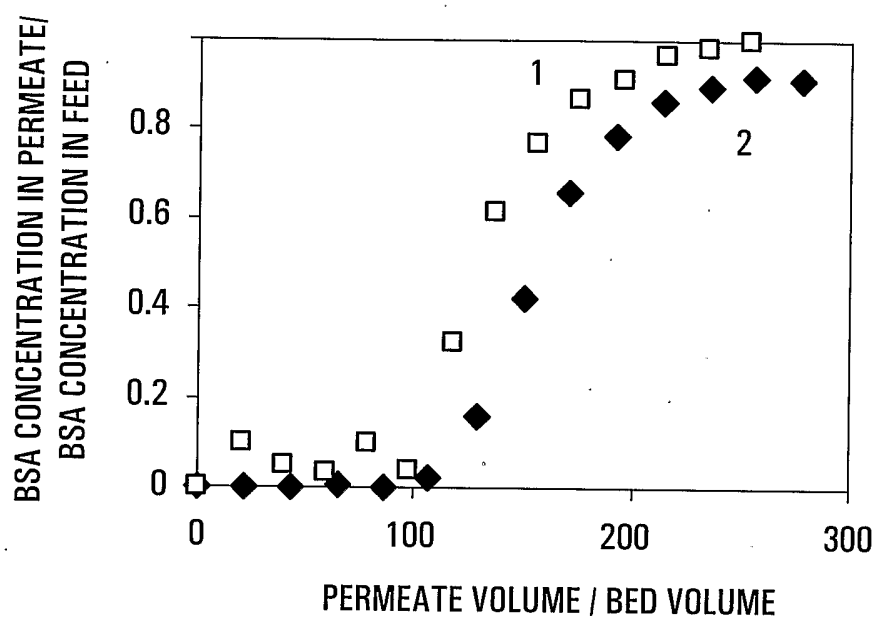
**FIG. 13**

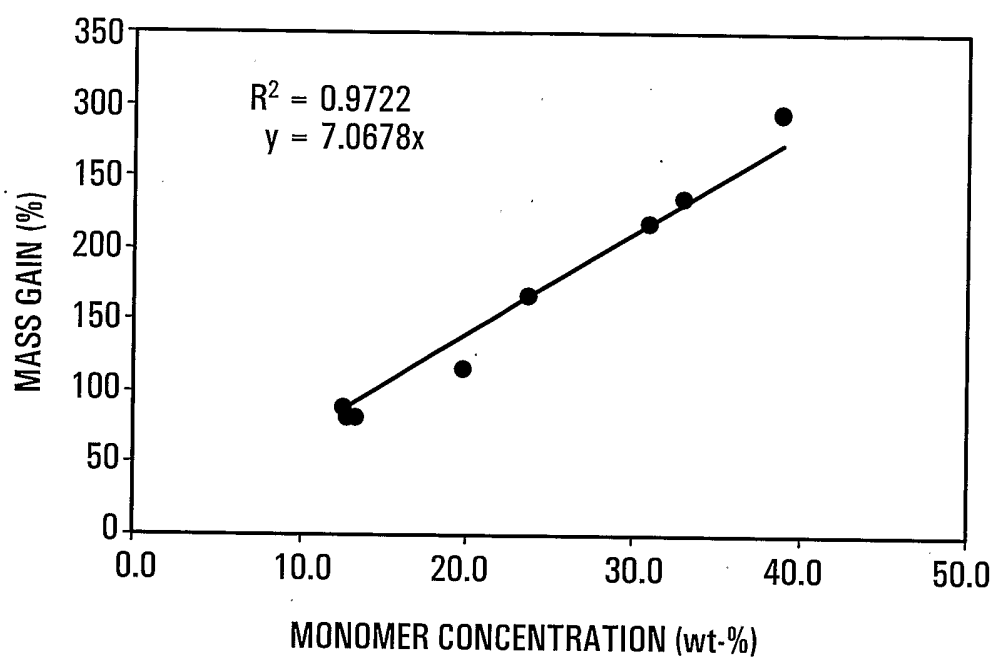
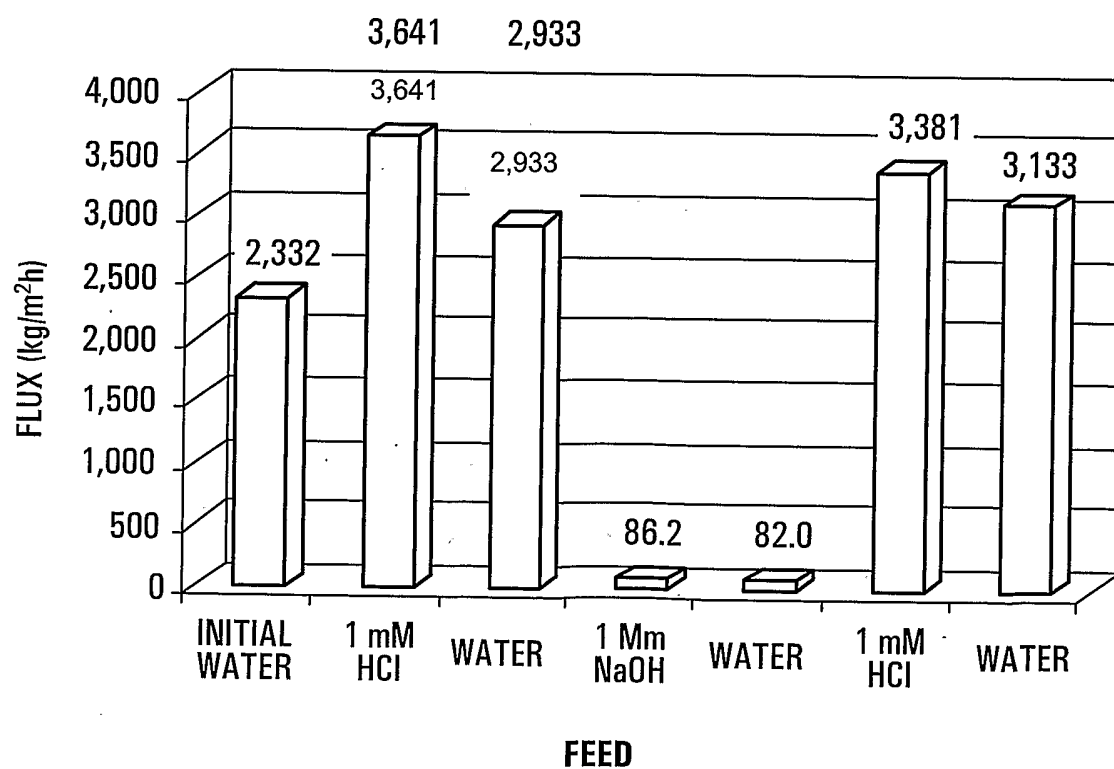


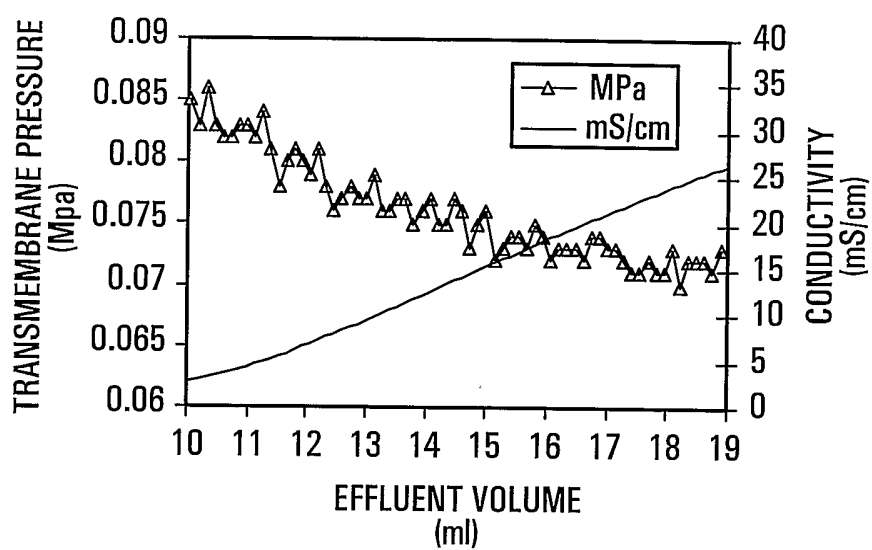
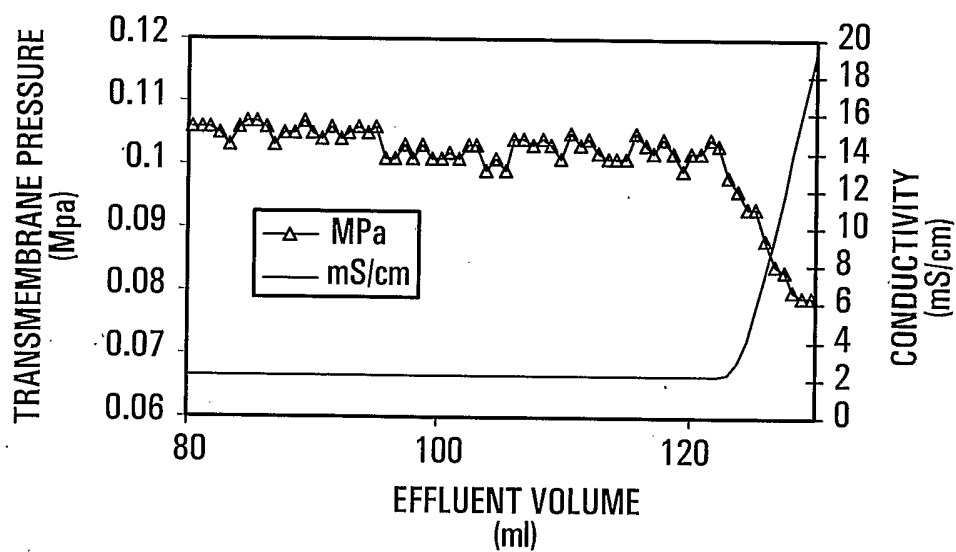
**FIG. 14**



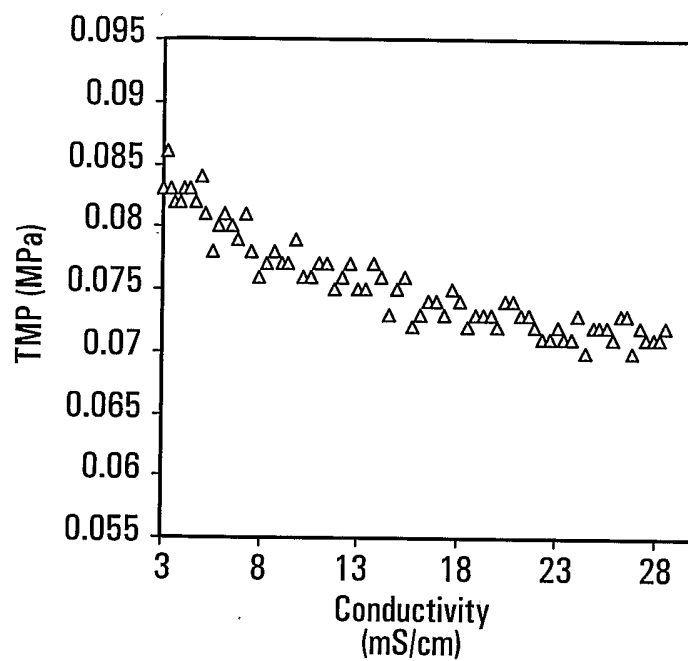
**FIG. 15**

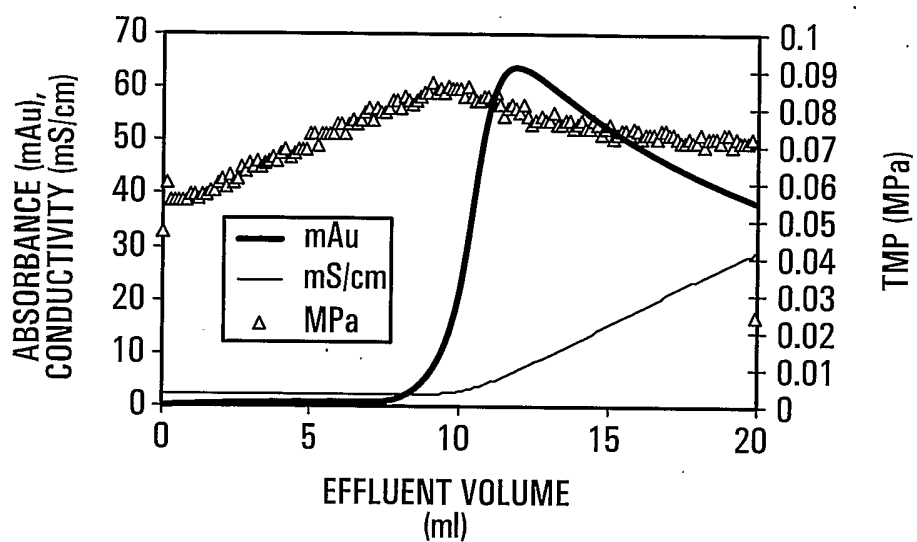
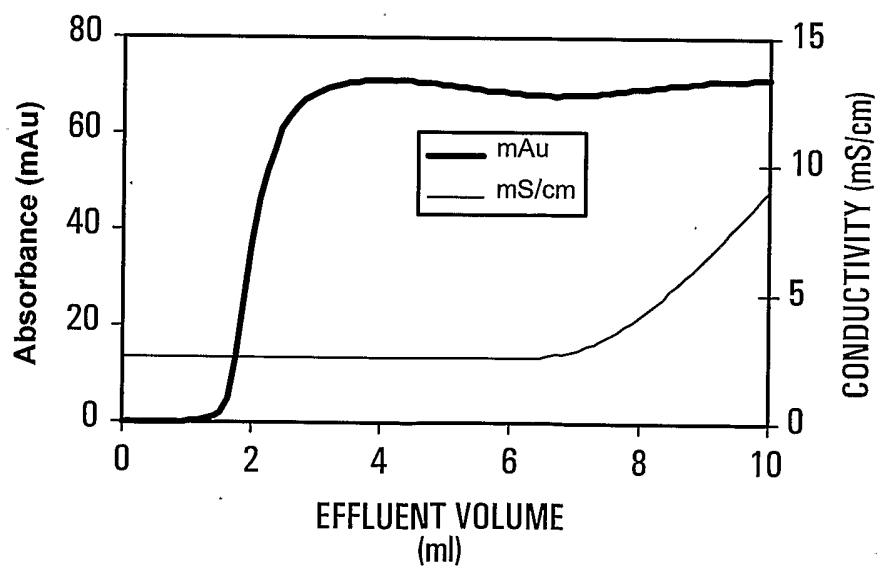
**FIG. 16**

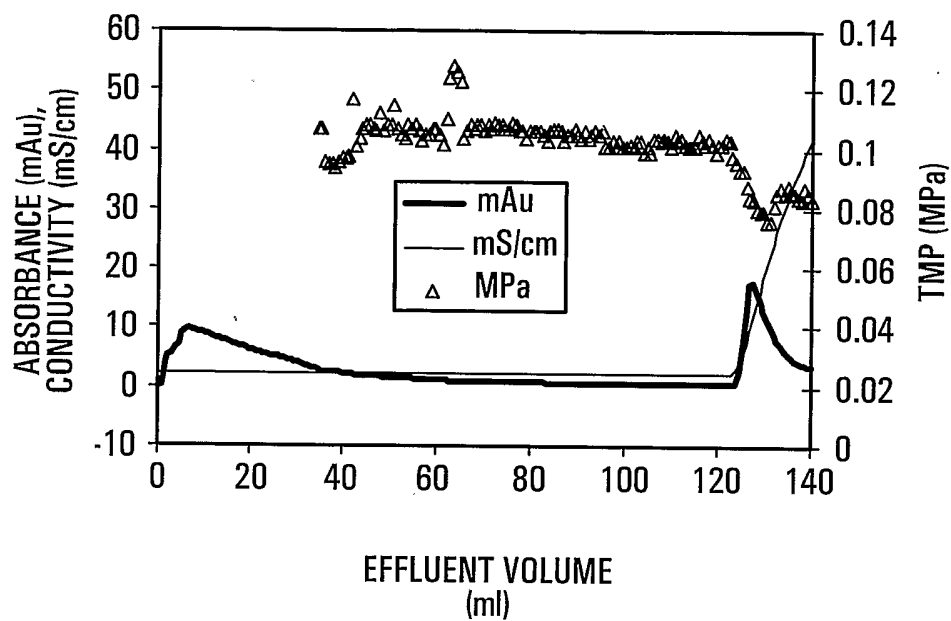
**FIG. 17****FIG. 18**

**FIG. 19A****FIG. 19B**



**FIG. 19C**

**FIG. 20****FIG. 21**

**FIG. 22**

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA2004/000120

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 B01D67/00 B01D69/10 B01D69/14 B01D69/12 B01D15/08  
B01J20/32 G01N30/48

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)

IPC 7 B01D B01J G01N

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 practical, search terms used)

EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/17129 A (UNIV TOLEDO) 15 May 1997 (1997-05-15)  the whole document	1-10, 12-18, 20-24, 26-30, 35, 36, 44-68
X	MIKA ALICJA, CHILDS RONALD, DICKSON JAME: "Chemical valves based on poly(4-vinylpyridine)-filled microporous membranes" JOURNAL OF MEMBRANE SCIENCE, vol. 153, no. 1, 3 February 1999 (1999-02-03), pages 45-56, XP002280492 page 46 -page 49; tables 1,3  -/--	1-8, 10-16, 20, 22, 31-35, 44-48, 58, 69

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

17 May 2004

Date of mailing of the international search report

03/06/2004

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Authorized officer

Marti, P

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA2004/000120

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/54866 A (FOSTER MILLER INC ;MENDUM THOMAS H E (US); GOLD HARRIS (US); HICKS) 21 September 2000 (2000-09-21)  page 11, line 26 -page 18, line 11 page 21, line 19 -page 24, line 7 page 25, line 18 -page 28, line 32; example 12  ---	1-6,10, 13-15, 18, 20-22, 31, 33-35, 44, 48-50, 53,69
P,X	JIANG W ET AL: "Pore-filled cation-exchange membranes containing poly(styrenesulfonic acid) gels" DESALINATION, ELSEVIER SCIENTIFIC PUBLISHING CO, AMSTERDAM, NL, vol. 159, no. 3, 5 November 2003 (2003-11-05), pages 253-266, XP004494865 ISSN: 0011-9164 the whole document  ---	1-6,10, 11, 13-17, 20,22, 35,37, 44-49
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA2004/000120

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 160 627 A (CUSSLER EDWARD L ET AL) 3 November 1992 (1992-11-03) cited in the application column 2, line 44 -column 3, line 17 column 3, line 33 -column 8, line 39; examples 1-5 ---	1-6, 38-42
A	KIM J H ET AL: "Rapid temperature/pH response of porous alginate-g-poly(N-isopropylac rylamide) hydrogels" POLYMER, ELSEVIER SCIENCE PUBLISHERS B.V, GB, vol. 43, no. 26, December 2002 (2002-12), pages 7549-7558, XP004393273 ISSN: 0032-3861 the whole document ---	1-68
A	LIU 1 Q ET AL: "Preparation of macroporous poly(2-hydroxyethyl methacrylate) hydrogels by enhanced phase separation" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 21, no. 21, 1 November 2000 (2000-11-01), pages 2163-2169, XP004216031 ISSN: 0142-9612 the whole document ---	1-30, 44-68
A	US 3 997 482 A (TURKOVA JAROSLAVA ET AL) 14 December 1976 (1976-12-14) the whole document -----	1-72

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CA2004/000120

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-34,44-68

Composite material comprising a support member having a plurality of pores and a macroporous cross-linked gel filling the pores of the support member; and process for preparing said composite material.

2. Claims: 35-36

Process for size-exclusion filtration which comprises passing a liquid through a composite material according to any of claims 1-34.

3. Claim : 37

Process for Donnan exclusion separation which comprises passing a liquid through a composite material according to any of claims 1-34.

4. Claims: 38-42

Process for adsorbing a biological molecule or a biological ion from a liquid, which comprises passing a liquid through a composite material according to claim 1.

5. Claim : 43

Process for solid phase chemical synthesis which comprises passing a liquid through a composite material according to any of claims 1-34.

6. Claims: 69-72

Process for chromatographic filtration of a solution or suspension containing two or more species of different size, which process comprises passing a liquid through a composite material according to claim 31.

The common inventive concept linking together all groups is a composite material comprising a support member having a plurality of pores and a macroporous cross-linked gel



filling the pores of the support member. Since this concept is not novel in view of the available prior art, the different subject-matter are not so linked as to form a single inventive concept. Therefore, the application lacks unity within the meaning of Rule 13 PCT.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/CA2004/000120

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