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(54) Title: CHROMATOGRAPHY MATERIALS, A PROCESS FOR THEIR PREPARATION AND USE OF THE MATERIALS		
(57) Abstract Products comprising a base matrix consisting of a copolymer of low molecular weight vinyl monomers and a polyhydroxy polymer, for example dextran, carrying vinylic groups on the polymer chain, which base matrix is modified with ion exchange groups. The products, which can be prepared by direct bonding of ion exchange groups, are useful in chromatographic separation processes based on ion exchange principles.		

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CHROMATOGRAPHY MATERIALS, A PROCESS FOR THEIR PREPARATION AND USE OF THE MATERIALS

The present invention relates to products which are useful in chromatographic separation processes based on ion exchange. The products comprise a base matrix consisting of a copolymer of low molecular weight vinyl monomers and water soluble macromolecules carrying vinylic groups on the polymer chain, which base matrix is modified with ion exchange groups. The invention also relates to a process for the preparation of the new products and to the use of the products in separation processes.

The above-mentioned chromatographic base matrices have been described in US-A-4,094,833 and one variant is sold under the name Sephacryl® (Amersham Pharmacia Biotech AB, Uppsala, Sweden). US-A-4,094,833 disclose that ion exchange groups can be linked to the part of the matrix derived from the lower molecular weight vinylic monomers. There are some publications disclosing that conventional affinity ligands linked to Sephacryl® via nucleophilic hydroxy groups of the base matrix have been used in affinity adsorptions based on specific interactions (e.g. Bisse et al, J. Chromatog. 575(2) (1992) 223-228; Bonde et al., Anal. Biochem. 200(1) (1992) 195-198; Algiman et al, J. Chromatog. 510 (1990) 165-175; Pepper, EP-A-209251).

Chromatographic processes for separation based on reversible adsorption/binding of a target substance to ion exchange groups attached to a porous base matrix in particle form are well known and extensively used. Such processes and materials are used both for analytical and preparative work in different fields, for example within the biochemical field for separation of proteins, nucleic acids etc. The base matrix material, which most often is in the form of spherical particles, can be inorganic, for example of glass or silica,

or based on different synthetic or natural organic polymers. In several widely used commercial products for both standard chromatography, i.e. based on size exclusion principles, and chromatography based on ion exchange, natural, hydrophilic
5 polymers, such as cellulose, agarose or dextran, are used as matrices, most often in cross-linked form to increase the physical stability.

Chromatography materials for ion exchange based separation processes should fulfil several important requirements, such
10 as a) high capacity, b) high recovery, c) rapid kinetics during adsorption and desorption (this gives the possibility of utilizing high flow rates and short beds/columns, i.e. short contact time), and d) permit adsorption of target substances, such as proteins, at one ion strength and
15 desorption at a higher ion strength or by change of pH possibly in combination with a lower ion strength. The materials should of course also have a suitable porosity and be physically and chemically stable and it should be possible to produce them by a technically comparatively simple process
20 and at a reasonable cost.

In comparison with chromatography materials having affinity groups including ion exchange groups bonded directly via simple ligand arms to a rigid base matrix, materials with increased capacity for proteins and other macromolecules have
25 been developed through the binding of affinity groups to flexible polymeric structures ("extenders") which in turn are bonded to the surfaces of the pores of base matrix. The increase in capacity is believed primarily to depend on an increased or higher availability of the affinity groups for
30 the target substance to be adsorbed than in conventional affinity supports. This increase in capacity can be obtained starting from a base matrix by binding the flexible extending polymeric structures to the base matrix and then binding the

affinity structures to these extending parts. Further, at least one additional reaction step of activating the surface of the base matrix in order to bind the extending polymeric structures is usually required. A chromatographic material
5 modified in this manner is for example disclosed in an article by Janzen, R. et al in Journal of Chromatography, 522 (1990) 77-93, which i.a. relates to materials prepared by subjecting pretreated glass beads and silica to a three step procedure.

10 According to the present invention it has been found that products which comprise a porous base matrix in particle form made up from a copolymer of a vinyl derivative of one or more polyhydroxy polymers and one or more low molecular weight vinyl compounds, which base matrix has ion exchange groups
15 bound to flexible polymeric parts of the vinyl substituted polyhydroxy polymer used extending into the pore volume, are extremely good ion exchange chromatography materials. They fulfil the above mentioned requirements and the products have a surprisingly high capacity for proteins. Further, the
20 products according to the invention can be produced by a technically and economically advantageous process, since the flexible polymeric structures (extenders) already are an integrated part of the base matrix. Thus no extra steps are required to introduce these parts of the product.

25 The present invention thus relates to novel products useful in chromatography processes as defined in the appended claims.

The "base matrix" of the products according to the invention is made up from "polyhydroxy polymers" carrying vinyl
30 substituents which polymers have been copolymerised with at least one vinyl compound, such as a divinyl compound. By "polyhydroxy polymers" is herein intended synthetic and natural polymers carrying a plurality of hydroxy groups. The

polyhydroxy polymers are water soluble and before copolymerisation they usually have weight average molecular weights of from 3000 and up to about 10 000 000 Dalton. As examples of synthetic such polymers can be mentioned
5 polyvinyl alcohol and partially hydrolysed polyvinyl acetate and as examples of natural polymers can be mentioned polysaccharides such as starch and cellulose which have been modified to suitable water solubility and agarose and dextran. Dextran is the preferred polyhydroxy polymer. It is
10 an important feature that the polyhydroxy polymer from which the base matrix is formed carries vinyl substituents.

The vinyl substituent can in principle be any substituent containing the vinyl grouping $\text{CH}_2=\text{CR}-$ in which R is hydrogen, methyl, halogen or cyano. Examples of substituents include
15 vinyl, allyl, methallyl, 3-allyloxy-2-hydroxypropyl and 2-chloroallyl. The most preferred vinyl substituents are allyl groups and 3-allyloxy-2-hydroxypropyl groups. The degree of vinyl substitution is suitably from 0.05 to 2 mmol/g polyhydroxy polymer and preferably from 0.1 to 1.5 mmol/g
20 polyhydroxy polymer.

The vinyl substituted polyhydroxy polymer is copolymerised with at least one vinyl compound such as a divinyl compound or a mixture of divinyl and monovinyl compounds to give a base matrix in the form of a porous, rigid gel. The divinyl
25 compound is of low molecular weight and as examples of suitable divinyl compounds can be mentioned N,N'-methylene-bisacrylamide, divinyl ketone and divinyl sulphone. N,N'-methylene-bisacrylamide is especially preferred. In order to be called low molecular weight vinyl compound, the molecular
30 weight shall typically be below 2000 dalton more often below 1000 dalton and contain no polyhydroxy polymer structure. The copolymerisation is carried out according to known free-radical polymerisation techniques under suitable conditions.

Usually an amount of vinyl substituted poly-hydroxy polymer in the range of 20 to 80% by weight is copolymerised with from 20 to 80% by weight of divinyl compound and 0 to 40% by weight of monovinyl compound. The rigid gel obtained by the
5 copolymerisation may be disintegrated to particles of desired size for the intended use but it is, of course, preferred to carry out the copolymerisation in such a manner that substantially spherical particles, beads, are formed directly by the utilized copolymerisation process. Such bead
10 polymerisation processes are well known to the man skilled in the art.

The base matrix of the products of the present invention is most preferably based on vinyl substituted dextran and in particular dextran with allyl substituents which has been
15 copolymerised with N,N'-methylene-bisacrylamide. Base matrices of this type are commercially available under the trademark Sephacryl® (Pharmacia Biotech AB, Sweden) which products are designed for size exclusion chromatography and have excellent properties with regard to porosity, chemical
20 and physical stability etc. These base matrices and processes for their preparation are disclosed in US-A-4,094,833, which is incorporated herein by reference. Base matrices based on other vinyl substituted polyhydroxy polymers than dextran can for example be prepared as disclosed in US-A-4,094,833.

25 The base matrix is in particle form, preferably in the form of beads, and usually has an average particle diameter within the range of from 10 to 1000 μm , preferably within the range of from 20 to 700 μm .

It has been found that base matrices prepared from a vinyl
30 substituted polyhydroxy polymer and a divinyl compound as a comonomer, e.g. as disclosed in US-A-4,094,833, will be built up as a porous, rigid, network structure which as integrated parts comprise flexible polymeric parts of the starting

polyhydroxy polymer, which have not been copolymerised into the network of the base matrix but extend from the pore surfaces of the base matrix into the pore volume. The flexible polymeric parts are thus in fact part of the base matrix, derived from the original starting polyhydroxy polymer and integrated with the rigid base matrix by being attached to this at one or more points and the flexible parts will be substantially non-cross-linked. The described flexible polymeric parts, which extend into the pore volume like arms or branches, are herein referred to as "extenders".

The present invention thus offers the possibility of an extremely simple preparation method for chromatography materials by direct bonding of ion exchange groups to existing extenders in a base matrix based on vinyl substituted polyhydroxy polymers copolymerised with a divinyl compound. Thus special steps such as surface activation and/or other coupling steps to attach extenders to a base matrix are avoided.

By "ion exchange groups" as the term is used herein is intended ligands or groups which have the capacity of attracting and reversibly adsorbing target substances that carry a charge of the opposite sign as ion exchange group on the support, for instance various types of nucleic acids, proteins, acids, amines etc. Typical ion exchange groups are: Positively charged groups, e.g. protonated forms of primary, secondary and tertiary amino groups, and quaternary amino groups (anion exchanging groups), and negatively charged groups: e.g. carboxy groups ($-\text{COO}^-/\text{COOH}$), phosphonate/phosphonic acid groups ($-\text{PO}_3^{2-}/-\text{PO}_3\text{H}^-/-\text{PO}_3\text{H}_2$), phosphate/phosphoric acid groups ($-\text{O}-\text{PO}_3^{2-}/-\text{O}-\text{PO}_3\text{H}^-/-\text{O}-\text{PO}_3\text{H}_2$), sulphonate/sulphonic acid groups ($-\text{SO}_2^-/-\text{SO}_2\text{H}$; sulfo groups), (cation exchanging groups).

The ion exchange groups can be chemically bonded to the

base matrix by methods known to the man skilled in the art for attaching this type of groups to matrices exhibiting hydroxy groups. Some of the methods are disclosed in the experimental part. So called linker arms are typically
5 introduced between the ion exchange groups (charged groups) and the extender.

It is preferred that the ion exchange groups in the chromatographic/separation material of the present invention are of the same kind as those expressly discussed above. As
10 examples of preferred ion exchange groups can be mentioned the protonated forms of diethylamino and trimethylamino, carboxy and sulfo groups, all of which being linked to the gel by the appropriate linker arm. Popular linker arms comprise alkane or hydroxy alkane structures typically being
15 interrupted by ether linkages and suitably having from 1 to 20 carbon atoms with preference for 1 to 6 carbon atoms. As another example of a suitable ion exchange group can be mention the protonated form of 2-[tris(hydroxymethyl)amino]-1-hydroxyethyl and of 2-[tris(hydroxymethyl)amino]-. The
20 products of the invention can be either strong or weak ion exchangers.

It is known to incorporate ion exchange groups in the base matrix material according to US-A-4,094,833 cited above. The only actual example shown in this reference is, however, a
25 polymerisation of allyl substituted dextran with a divinyl compound and additionally a charged comonomer. In this case the charged comonomer will form part only of the rigid base matrix and not be attached to extenders and the high capacity observed when ion exchange groups are bound to extenders will
30 not be obtained in this case.

The novel products of the invention have ion exchange groups bonded to hydroxyl groups on the polyhydroxy polymer in the rigid matrix and especially on the extenders, which

are parts of the polyhydroxy polymer of the base matrix. They may have a total degree of substitution with respect to ion exchange groups (or ion exchange capacity) in the interval from 10 to 500 with preference for 100 to 350 $\mu\text{mol/ml}$ gel.

5 The products will of course have some ion exchange groups attached to hydroxyl groups in the rigid base matrix, both on the outer particle surfaces and the pore surfaces, but the ion exchange groups will predominantly be attached to the extenders. The products of the present invention fulfil the

10 requirements for chromatography materials mentioned earlier in this specification to a very high extent and in particular they show a very high dynamic binding capacity for target substances, such as proteins, that are prone to be adsorbed by matrices carrying positively and/or negatively charged

15 groups. The dynamic binding capacity can be said to be a measure of the obtainable productivity. The fact that the groups are bonded to extenders give an extremely rapid adsorption or binding of target molecules.

As mentioned above, the products of the present invention

20 are advantageously and preferably prepared by direct introduction/bonding of ion exchange groups to existing extenders in a base matrix based on vinyl substituted polyhydroxy polymers copolymerised with a vinyl compound, such as a divinyl compound, to a degree of substitution of

25 ion exchange groups in the range of from 10 to 500 $\mu\text{mol/ml}$ gel. It might also be possible to produce the products of the invention by utilizing as starting material for copolymerisation with vinyl compounds a vinyl substituted polyhydroxy polymer exhibiting the ion exchange groups.

30 The chromatography products of the present invention can be used in the different modes of separation/chromatography processes, such as in packed beds, fluidized beds or stirred suspensions according to per se well known techniques. The

products of the invention can function for separation of compounds of various molecular weights and types. Some examples are macromolecules, e.g. with molecular weights of from about 5000 Dalton, such as polysaccharides, 5 proteins/polypeptides and nucleic acids and synthetic water soluble polymers. Also substances with molecular weights lower than 5000 may be separated by use of the present products. The products of the invention can for example be used in treatment of processed and unprocessed supernatants/cul- 10 ture media from fermentors and other cell culture vessels, serum, plasma, beverages etc. Either the target substance which is adsorbed or the sample from which it is adsorbed is then further processed.

It is particularly preferred to use the present products in 15 separations based on packed bed chromatography with plug flow in order to take advantage of the excellent properties the products of the invention have. A specific advantage is here that a high capacity can be obtained with fairly large particles, which is advantageous with regard to flow rate. 20 The products of the invention are further preferably used in treatment/separation of macromolecules, as above, and especially for adsorption/binding of proteins.

The invention is further illustrated in the following examples which, however, are not intended to limit the same. 25 Parts and per cent relate to parts by weight and per cent by weight unless otherwise stated.

EXPERIMENTAL PART

PART 1. Synthesis

30 **Example 1. Synthesis of a base matrix.**

75 g of fine-grade chalk coated with stearate, 2.0 g anionic surfactant (organic phosphate ester) and 1000 ml heptane were charged in a round bottomed flask. The mixture

was heated to 50°C.

35.0 g of allyldextran ($M_w = 2,000,000$ and degree of substitution 1.40 mmol allyl groups per g of dextran derivative) and 75.0 g of N,N'-methylene-bisacrylamide were dissolved in
5 a mixture of 250 ml of distilled water and 250 ml of methanol at 50°C. When a clear solution had been obtained, 0.5 g of ammonium persulphate was added. The water-methanol solution and heptane mixture were brought together and stirred for 10 minutes at 50°C in a nitrogen gas atmosphere. 0.36 ml of
10 N,N,N',N'-tetramethylethylenediamine was added and the reaction was continued for 2 hours at 50°C. Insoluble gel beads having a size of 40-300 μm were obtained. The product was washed with acetic acid, ethanol and distilled water. Subsequent to sedimentation, the yield was measured to 810 ml.

15

Example 2. Synthesis of a quaternary ammonium exchanger.

The base matrix beads from Example 1 were drained by suction on a glass filter, and 100.0 g of beads were added to a reaction vessel. 4.0 g sodium hydroxide and 0.1 g sodium
20 borohydride were stirred with 20 ml distilled water to a clear solution and charged to the reaction vessel. 90 ml glycidyltrimethylammonium chloride (GMAC) was pumped into the reaction vessel in 2 hours. The temperature was kept at 25°C and the reaction continued during the night (18 hours).

25 The product was neutralised with acetic acid and washed with distilled water.

The amount of ion exchanger groups was determined by the following method. 1 to 3 ml of the beads were sedimented in a PD-10 column (Amersham Pharmacia Biotech AB, Uppsala, Sweden)
30 and the exact volume was determined. The column was eluted with 10 ml of 0.5 M hydrochloric acid and washed with 1 mM hydrochloric acid. The gel was transferred to a titration cup with 10 ml of distilled water and 1 drop of concentrated

nitric acid was added. Titration was finally done with 0,1 M silver nitrate (Mettler titrator). The result was 169 $\mu\text{mol Cl}^-/\text{ml gel}$.

5 Example 3. Synthesis of a quaternary ammonium exchanger.

An ion exchanger was prepared in a manner analogous with Example 2 but with 20 ml of GMAC. The ion capacity of the product was 72 $\mu\text{mol Cl}^-/\text{ml gel}$.

10 Example 4. Synthesis of a quaternary ammonium exchanger.

An ion exchanger was prepared in a manner analogous with Example 2 but with 40 ml of GMAC. The ion capacity was 116 $\mu\text{mol Cl}^-/\text{ml gel}$.

15 Example 5. Synthesis of a quaternary ammonium exchanger.

An ion exchanger was prepared in a manner analogous with Example 2 but with 200 ml of GMAC. The ion capacity was 220 $\mu\text{mol Cl}^-/\text{ml gel}$.

20 Example 6. Synthesis of a sulphopropyl cation exchanger.

100.0 g base matrix (Ex. 1) beads drained by suction on a glass filter were mixed with 26 ml distilled water and 15.0 g sodium bisulphite. Aqueous 45% sodium hydroxide was added during stirring until the pH was 6.8. The temperature was 25°C and air was bubbled into the reaction vessel through a capillary. The reaction continued during stirring overnight at 25°C. The product was washed with distilled water.

The amount of sulfonate groups bound to the beads was determined by the following method. A sample consisting of 1 to 3 ml of the beads was sedimented in a PD-10 column (Amersham Pharmacia Biotech AB, Sweden) and the exact volume of the beads was determined by measuring the height and diameter of the sediment in the column. The column was eluted

with 10 ml of 0.5 M hydrochloric acid and washed with 1 mM hydrochloric acid. The beads were transferred to a titration cup with 10 ml of distilled water and titrated with 0.1 M sodium hydroxide (Mettler titrator). The observed consumption
5 of sodium hydroxide corresponds to the presence of 134 μmol SO_3^- groups per ml gel.

**Example 7. Two-step synthesis of a sulphopropyl cation exchanger by bisulphite coupling to vinyl groups remaining
10 after polymerisation and subsequent allylation and a second bisulphite coupling.**

In a manner analogous with Example 6 a bisulphite coupling to the base matrix (Ex. 1) was done. The content of sulfonate groups (SO_3H) was 141 $\mu\text{mol}/\text{ml}$ gel.

15 Allylation of the resulting beads was done in the following way. 100.0 g beads drained by suction on a glass filter and containing 141 μmol $\text{SO}_3\text{H}/\text{ml}$ were charged to the reaction vessel. 4.0 g sodium hydroxide. 0.4 g sodium borohydride, 13.0 g sodium sulphate and 50 ml distilled water were stirred
20 to a clear solution and added to the reaction vessel. The temperature was 35°C and finally 40 ml allylglycidylether was added during stirring. The reaction continued overnight and the product was neutralised with acetic acid and washed with distilled water.

25 The amount of allyl groups was determined by the following method. The volume of the sample was measured in a PD-10 column. The beads were transferred with 10 ml of distilled water to a 100 ml filter flask, and during stirring bromide water was added until a permanent yellow colour remained.
30 Then vacuum was applied under stirring until the mixture was colourless. The sample was now transferred to a titration cup with 10 ml of distilled water and 1 drop of concentrated nitric acid was added. Titration was done with 0,1 M silver

nitrate and the result was 151 μmol allyl groups per ml of gel.

Bisulphite coupling of the allylated beads above was done in a manner analogous with Example 6, and the content of 5 SO_3H was 249 $\mu\text{mol}/\text{ml}$ gel, determined as in example 6.

Example 8. Synthesis of a diethylaminoethyl anion exchanger.

21.0 g sodium sulphate, 6.0 g sodium hydroxide and 0.2 g sodium borohydride were stirred with 60 ml of water in a 10 reaction vessel to a clear solution and 100.0 g of beads, drained by suction on a glass filter, of base matrix (Ex. 1) was added. 50.0 g of 2-diethylaminoethyl chloride hydrochloride (65 % solution) was added and the mixture was stirred at 35°C. After one hour the pH was 6.2 and 6.0 g 15 sodium hydroxide were charged. After 15 minutes the pH was 12.4 and additionally 2.0 g sodium hydroxide was added causing the pH to rise to 12.6. The reaction mixture was stirred overnight. The product was neutralised with acetic acid and washed with distilled water.

20 The amount of ion exchanger groups was determined in a manner analogous with Example 2. The result was an ion capacity of 267 $\mu\text{mol Cl}^-/\text{ml}$ gel.

Example 9. Synthesis of a diethylaminoethyl anion exchanger.

25 An anion exchanger was prepared in a manner analogous with Example 8 but with 20.0 g 2-diethylaminoethyl chloride hydrochloride and 25°C during the reaction. The ion capacity was 75 $\mu\text{mol Cl}^-/\text{ml}$ gel.

30 **Example 10. Synthesis of a diethylaminoethyl anion exchanger.**

An anion exchanger was prepared in a manner analogous with Example 8 but with 20.0 g 2-diethylaminoethyl chloride hydrochloride and 35°C during the reaction. The ion capacity

was 121 $\mu\text{mol Cl}^-/\text{ml}$ gel.

PART 2. DETERMINATION OF THE DYNAMIC PROTEIN BINDING CAPACITIES, TOTAL PROTEIN CAPACITIES AND PROTEIN RECOVERIES OF THE ION EXCHANGERS.

Instruments.

All the instruments used were manufactured by Amersham Pharmacia Biotech AB, Sweden.

10 The chromatographic equipment used was FPLC® System equipped with the controller unit LCC-501 plus with the software FPLC director®, two P-500 pumps, one MV-7 and two MV-8 valves and the monitors Monitor UV-M and Conductivity Monitor.

15

Buffers

Buffer A 50 mM tris, pH 8.0 (anion exchangers)

50 mM glycine, pH 9.0 (cation exchangers)

Buffer B 50 mM tris, 1 M NaCl, pH 8.0 (anion exchangers)

20 50 mM glycine, 0.5 M NaCl, pH 9.0 (cation exchangers)

Packing of the column.

The ion exchangers were packed with suction in HR 5/5 columns and washed with at least 10 volumes of buffer A (buffer without salt), with a flow rate of 1200 cm/hour.

About 1 ml of gel was packed in a column.

Comparison between ion exchangers.

One of the most used media in ion exchange chromatography of proteins is Sepharose® Fast Flow. Sepharose® Fast Flow is a chromatographic support consisting of porous, spherical particles of highly cross-linked agarose (from Amersham Pharmacia Biotech AB, Sweden). A comparison between the

present ion exchangers and Sepharose® Fast Flow ion exchangers was made as follows.

The flow rate in the test in which the dynamic binding capacity ($Q_{B,10\%}$) was determined was 300 cm/h. $Q_{B,10\%}$ represents
5 the amount of protein fed to the column, when A_{280} in the eluate is 10 % of A_{280} in the protein solution fed to the column. A_{280} stands for the absorbance of UV-light at the wave length of 280 nm.

The test protein was dissolved in buffer A (0,2 %), and the
10 ion exchanger was saturated with protein during 3 hours. Then the ion exchanger was washed with A-buffer during 1 hour and finally the test protein was eluted during 1 hour with buffer B.

Anion exchangers were tested with bovine serum albumin
15 (BSA) and the buffer was 50 mM tris pH 8.0. The albumin was eluted from the ion exchanger with 1 M sodium chloride in the buffer.

Cationic exchangers were tested with lysozyme and the
buffer 50 mM glycine pH 9.0. Lysozyme was eluted from the ion
20 exchanger with 0.5 M sodium chloride in the buffer.

RESULTS.***Quaternary ammonium ion exchanger.***

Ex. no	Ion exchange capacity ($\mu\text{mol Cl}^-/\text{ml gel}$)	Bead size (μm)	$Q_{B,10\%}$ (albumin/ ml gel)	Total BSA capacity (mg/ml gel)	Recovery of protein (%)
5					
3	72	160-315	81	91	86.9
4	116	160-315	131	150	93.8
10	2	160-315	159	189	96.1
5	220	160-315	153	185	102
Q Sepharose [®]					
Fast Flow	180-250	40-160	26		90.2

- 15 The dynamic binding capacity $Q_{B,10\%}$ of the ion exchanger of Example 2 is higher by a factor 6 ($=159/26$) than that of Q Sepharose[®] Fast Flow, despite the difference in particle size.

20 Sulphopropyl ion exchanger.

Ex. no	Titration with NaOH (μmol OH/ml gel)	Q _{B,10} , lysozyme (mg/ml gel)	Total lysozyme capacity (mg/ ml gel)	Recovery of protein (%)
25				
6	134	182	183	98.7
7	249	167	230	103
SP Sepharose [®]				
30	Fast Flow	180-250	110	149
				93.7

- All The SP ion exchangers were in the size of 40-160 μm . The increase of $Q_{B,10\%}$ with the ion exchanger according to the invention was $167/110=1.5$ as compared to SP Sepharose[®] Fast Flow.
- 35

Diethylaminoethyl ion exchanger

Ex. no	Ion exchange capacity ($\mu\text{mol Cl}^-/\text{ml gel}$)	$Q_{B,10\%}$ (albumin/ml gel)	Total BSA capacity (mg/ml gel)	Recovery of protein (%)
5				
8	267	120	139	71.5
9	75	67	79	74.0
10	121	96	136	86.0
10 DEAE Sepharose [®]				
Fast Flow	110-160	39	94	86.7

All the DEAE ion exchangers were in the size of 40-160 μm . The increase of $Q_{B,10\%}$ with the ion exchanger according to the invention in Example 8 was $120/39=3.1$.

PART 3. Rigidity of the base matrix.

A comparison was made between the rigidity of Sepharose[®] Fast Flow and a quaternary ion exchanger prepared according to the invention. A fraction of a base matrix prepared in a manner analogous to Example 1 but with a smaller particle size was used as starting material. The base matrix was sieved using 40 and 160 μm sieves. The product was modified to a quaternary ion exchanger analogous to Example 2. The gel was packed at a flow rate of 1 ml/min in an HR 10/30 column (Amersham Pharmacia Biotech AB, Sweden). The testing was done by increasing the flow rate through the column, keeping the flow rate constant for a fixed period of time at each step. The pressure drop over the system was recorded using the built-in recorder in FPLC System.

For comparison the same experiment was done with Q Sepharose[®] Fast Flow. The particle size is similar to that of the 40-160 μm fraction of the ion exchanger prepared according to the invention.

The result was that the Q ion exchanger according to the

invention can withstand higher flow rates than Sepharose® Fast Flow.

The collapse point of Sepharose Fast Flow was reached at around 16 ml/min. The collapse point for the ion exchanger prepared according to the invention had not been reached at flow rate of 20 ml/min.

PART 4. Chromatographic experiment

A quaternary ion exchanger and a diethylaminoethyl anion exchanger prepared as described in Example 2 and in Example 8 respectively were in two separate sets of experiments used as substitutes for Q Sepharose® Fast Flow in a process for purification of IgG from blood plasma.

In both cases the ion exchangers prepared according to the invention could be loaded with three times as much of protein as Q Sepharose® Fast Flow with retained high purity of the final IgG product.

CLAIMS

1. A product suitable for use in chromatography processes comprising a porous, rigid base matrix gel in particle form, **characterized** in that the base matrix is made up
5 from a vinyl substituted, water soluble polyhydroxy polymer which has been copolymerised with a low molecular weight vinyl compound, such as a divinyl compound, said base matrix having flexible polymeric parts of the polyhydroxy polymer attached to the base matrix and
10 integrated with this, which flexible polymeric parts (extenders) extend into the pore volume and in that the hydroxy groups of the polymer are substituted with ion exchange groups to a total degree of substitution of from 10 to 500 $\mu\text{mol/ml}$ gel.
15
2. A product according to claim 1, **characterized** in that the polyhydroxy polymer is selected from the group consisting of polyvinyl alcohol, partially hydrolysed polyvinyl acetate, water soluble modified starch or cellulose,
20 agarose and dextran.
3. A product according to claim 2, **characterized** in that the polyhydroxy polymer is dextran.
- 25 4. A product according to any of claims 1 to 3, **characterized** in that the vinyl substituent on the polyhydroxy polymer is an allyl group or a 3-allyloxy-2-hydroxypropyl group.
- 30 5. A product according to claim 1, **characterized** in that the vinyl compound is a divinyl compound which is N,N'-methylene-bisacrylamide.

6. A product according to anyone of claims 1-5, **characterized** in that the ion exchange groups are diethylamino, trimethylamino, carboxy or sulfo groups linked to the gel via alkane or hydroxy alkane groups, optionally containing ether linkages.
7. A product according to claim 1, **characterized** in that the base matrix is made up from a vinyl substituted dextran which has been copolymerised with a divinyl compound, said base matrix having extenders of the vinyl substituted dextran and in that the hydroxy groups of the dextran are substituted with ion exchange groups to a total degree of substitution of from 10 to 500 $\mu\text{mol/ml}$ gel and in that the product is in the form of beads having an average diameter of from 10 to 1000 μm .
8. A product according to claim 7, **characterized** in that the vinyl substituent is an allyl group or a 3-allyloxy-2-hydroxypropyl group.
9. A product according to any one of claims 7-8, **characterized** in that the ion exchange groups are amino groups, such as diethylamino or trimethylamino; or carboxy; or sulfo groups linked to the gel via alkane or hydroxy alkane groups, optionally containing ether linkages.
10. A process for preparing a product as defined in any of claims 1 to 10, **characterized** in that a base matrix made up from a vinyl substituted, water soluble polyhydroxy polymer which has been copolymerised with a vinyl compound, such as a divinyl compound, said base matrix having flexible polymeric parts of the polyhydroxy

polymer attached to the base matrix and integrated with this, which flexible polymeric parts (extenders) extend into the pore volume, is reacted with a compound introducing ion exchange groups to a total degree of substitution in the interval from 10 to 500 $\mu\text{mol/ml}$ gel.

11. A process for preparing a chromatography product, **characterized** in that a base matrix made up from vinyl substituted, preferably allyl or 3-allyloxy-2-hydroxypropyl substituted, dextran which has been copolymerised with a divinyl compound, preferably N,N'-methylene-bisacrylamide, said base matrix having flexible polymeric parts of the dextran attached to the base matrix and integrated with this, which flexible polymeric parts (extenders) extend into the pore volume, said base matrix with extenders having been prepared by copolymerisation of vinyl substituted, preferably allyl substituted, dextran and a divinyl compound, preferably N,N'-methylene-bisacrylamide, is reacted with a compound introducing ion exchange groups to a total degree of substitution in the interval from 10 to 500 $\mu\text{mol/ml}$ gel.

12. A process according to anyone of claims 10-11 **characterized** in that the ion exchange groups are amino groups, such as diethylamino or trimethylamino; carboxy; or sulfo groups linked to the gel via alkane or hydroxy alkane groups, optionally containing ether linkages.

13. Use based on ion exchange principles of a product as defined in any of claims 1-9, as chromatographic separation material.

14. Use according to claim 13, for separation of macromolecules.

15. Use according to claim 14, for separation of proteins.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01213

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: B01J 20/22, B01D 15/08, C08B 37/00, C08F 251/00
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)

IPC6: B01D, B01J, C08B

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

SE,DK,FI,NO classes as above

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4094833 A (HALDOR INGEMAR JOHANSSON ET AL), 13 June 1978 (13.06.78), column 2, line 30 - line 43; claims; abstract; example 10 --	1-15
X	EP 0209251 A2 (THE COMMON SERVICES AGENCY FOR THE SCOTTISH HEALTH SERVICE), 21 January 1987 (21.01.87), page 5, line 10 - line 22; page 8, line 26 - line 31; example 1, 11 --	1-15
A	WO 9708298 A1 (GENZYME CORPORATION), 6 March 1997 (06.03.97), page 5, line 10 - page 6, line 5; page 13, line 21 - line 34 --	1-15

☒ Further documents are listed in the continuation of Box C.☒ See patent family 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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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01213

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 8303363 A1 (RIESNER, DETLEV), 13 October 1983 (13.10.83), page 4, line 13 - line 26 -- -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.

PCT/SE 98/01213

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EP 0209251 A2	21/01/87	NONE	
WO 9708298 A1	06/03/97	AU 7010896 A EP 0847442 A	19/03/97 17/06/98
WO 8303363 A1	13/10/83	DE 3211309 A,C EP 0104210 A,B US 4699717 A	29/09/83 04/04/84 13/10/87