

MEDIA FOR MEMBRANE ION EXCHANGE CHROMATOGRAPHY BASED ON  
POLYMERIC PRIMARY AMINES, SORPTION DEVICE CONTAINING THAT MEDIA,  
AND CHROMATOGRAPHY SCHEME AND PURIFICATION METHOD USING THE SAME

This application is a divisional of U.S. Patent Application No. 13/167,291 filed June 23, 2011 which is a divisional of U.S. Patent Application No. 12/221,496 filed on August 14, 2008, claims priority of U.S. Provisional application serial no. 60/964,653 filed August 14, 2007 and U.S. Provisional application serial no. 61/070,708 filed on March 25, 2008, the disclosures of which have been incorporated herein by reference.

BACKGROUND OF THE INVENTION

This invention relates to anion exchange chromatography media based on polymeric primary amines, an anion exchange sorber including that media, and a chromatography scheme including the sorber. Absorption refers to taking up of matter by permeation into the body of an absorptive material. Adsorption refers to movement of molecules from a bulk phase onto the surface of an adsorptive media. Sorption is a general term that includes both adsorption and absorption. Similarly, a sorptive material or sorption device herein denoted as a sorber, refers to a material or device that either adsorbs or both adsorbs and absorbs. The media is particularly applicable as a porous membrane sorber used in a flow through cartridge and more particularly to a cartridge free of a separate exterior housing.

Strong anion exchangers, such as those based on quaternary ammonium ions, are used in downstream processing as a polishing media for capturing negatively charged large impurities, such as

endotoxins, viruses, nucleic acids, and host cell proteins (HCP)  
that are present in fluids such as biological fluids,

### Example 8

0.65 $\mu$ m UPE membrane rolls were coated with 9% PAA and 0.35% PEGDGE on the pilot coating machine. The membranes were extracted with water and methanol and dried on a hot air impingement dryer at 120°C. The rolls were then subjected to curing at varying temperatures and times as shown in the table below. The membranes were then treated with 5% sulfamic acid in 5% isopropyl alcohol to stabilize the coating. The permeability of the membranes was measured using a flux tester as described in the previous example.

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As seen in the table below, flow-times on the order of 50s can be obtained by heating the membrane to temperatures greater than 90°C for 12hours or longer. In case membranes with lower permeability (higher flow times on the order of 100s) are desired, the temperature can be limited to below 85°C and for shorter times (<10h).

| Cap, Flowtime<br>(mg/ml,s) | 8 hrs       | 10hrs | 12hrs     | 20hrs | 24hrs     |
|----------------------------|-------------|-------|-----------|-------|-----------|
| 85°C                       | 107.7,147.6 |       | 95,63.9   |       | 92.9,88.0 |
| 90°C                       |             | 95,41 |           | 90,39 |           |
| 95°C                       | 92.6,79.2   |       | 89.6,50.2 |       | 85.8,67.1 |
| 100°C                      |             | 79,39 |           | 63,41 |           |

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What is Claimed is:

1. A method of purifying a biological sample comprising antibodies and impurities, said method comprising: purifying said biological sample by subjecting said sample to affinity chromatography; further purifying said sample by subsequently loading said sample on anion exchange media comprising a porous coated media comprising a porous substrate and a porous coating on a surface of said porous substrate, said coating comprising one or more crosslinked polymeric primary amines or copolymers thereof; and collecting the further purified sample.
2. A method of purifying a biological sample comprising antibodies and impurities, said method comprising: purifying said biological sample by subjecting said sample to cation exchange chromatography; further purifying said sample by subsequently loading said sample on anion exchange media comprising a porous coated media comprising a porous substrate and a porous coating on a surface of said porous substrate, said coating comprising one or more crosslinked polymeric primary amines or

~~copolymers thereof; and collecting the further purified~~  
sample.

3. The method of claim 1, further comprising carrying out cation exchange after said affinity chromatography step and prior to loading said sample on said anion exchange media.
4. The method of claim 1, wherein said biological sample is a cell culture.
5. The method of claim 1, wherein said further purified sample is collected in the flow-through of said anion exchanger.

What is Claimed is:

1. A system for the purification of biological solutions, comprising an affinity chromatography separator, at least one cation exchanger, and an anion exchanger downstream of said cation exchanger, said anion exchanger comprising a porous coated media comprising a porous substrate and a porous coating on a surface of said porous substrate, said coating comprising one or more crosslinked polymeric primary amines or copolymers thereof.
2. The system of claim 1, wherein said media is positively charged.
3. The system of claim 1, wherein said affinity chromatography separator comprises a Protein A affinity column.
4. The system of claim 1, wherein said substrate comprises a non-woven material.
5. The system of claim 4, wherein said non-woven material is a mat or fabric.