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Membrane chromatography for rapid purification of recombinant antithrombin III and monoclonal antibodies from cell culture supernatant

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ABSTRACT

The task of purifying monoclonal antibodies (MAbs) and human recombinant antithrombin III (rATIII) from cell culture supernatant was carried out using two different approaches, both based on the use of membraneous matrices. The first approach employed a strongly acidic and a strongly basic membrane ion exchanger, which were evaluated for their ability to purify monoclonal antibodies and the human active recombinant antithrombin III from cell culture supernatant. Within minutes gram amounts of product could be purified in a high-flux system, specially developed for this purpose, achieving purities of 80% for MAbs and 75% for rATIII, respectively. The capacity of the acidic membrane ion exchanger for MAbs was found to be 1 mg/cm^2 with recoveries up to 96% and that of the basic membrane ion exchanger for rATIII was 0.15 mg/cm^2 with recoveries up to 91%. The second approach consisted of using heparin, a mucopolysaccharide with a strong affinity towards ATIII, coupled to amine-modified or epoxy-activated membranes by reductive amination, for the purification of rATIII. The ATIII binding capacities of the membranes were found to be 91 μg/cm^2 for the amine-modified and 39 μg/cm^2 for the epoxy-activated membrane, achieving purities of 75%. The coupling proved to be fairly stable over a period of 5 months and the membranes remained operable even after steam sterilization and treatment with sodium dodecyl sulphate. Final purification in both instances was carried out by gel filtration.

INTRODUCTION

Ion-exchange and affinity chromatography are common techniques for the purification and preparation of proteins, peptides and enzymes. However, using classical soft gels or porous particle media as a matrix results in a limitation arising from the restriction of mass transport in these diffusion-dominated systems [1,2]. A different approach is the attachment of functional groups to the inner surfaces of synthetic microporous membranes (Table I). The main advantage of using membranes as a matrix is that the mass transport is convective and therefore avoids the aforementioned limitation of classical matrices [1,2]. Other interesting features in addi-

tion to the high flow-rate include high adsorption rates and binding velocities, no problems arising from channelling, bed shift or bed collapse and the ease of scale-up and the comfort of using a prepacked stabilized matrix. Kroner and co-workers [1,2] showed the purification of monoclonal antibodies (MAbs) using protein A/G and acidic membrane ion exchangers on an analytical scale (single sheets of 13.4 and 42 cm^2), using the same type of membranes that are discussed in this investigation. The isolation of microbial enzymes with affinity membranes was described by Champluvier *et al.* [3], Briefs and Kula [4] and Kroner *et al.* [5].

The aim of this work was to test new prototypes of membrane ion exchangers and heparin affinity membranes. Analytical-scale purification was carrried out using a 100 cm^2 membrane in a fast protein liquid chromato-

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		syringe top	filter holder	membrane module	pleated module
Area: (cm^2)		5.4	12.56	100	1400, 2400
Flow-rate: (ml/min)		6 *	10 *	30 *	850 **

* FPLC, ** High flux system

Fig. 1. Schematic diagrams of the different housings containing the membranes. The syringe top and the membrane holder were equipped with single membrane sheets. The 100-cm^2 unit consisted of a stack of five membrane sheets. These three units were used with an FPLC or LC system. The membrane in the preparative-scale ion exchanger units is pleated, and was used with the HFS.

graphic (FPLC) system. For preparative-scale purification, 1400 and 2400 cm^2 membrane ion exchanges were used (Fig. 1).

A special high-flux system (Fig. 2) was developed for optimum use of the preparative membrane ion exchanger units (MIEX Q, S). This system allows high flow-rates (up to 1200
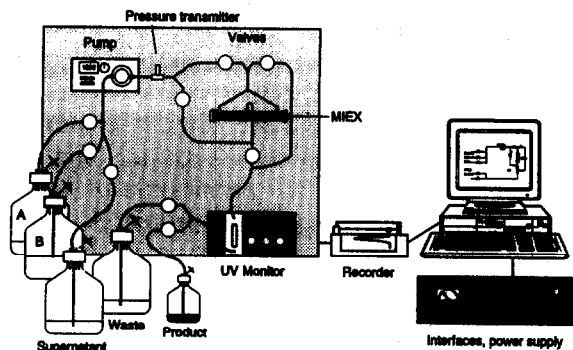

Fig. 2. High-flux system (HFS) consisting of a gear-wheel pump, a UV monitor, a pressure transmitter and special valves. A personal computer was equipped with interfaces for A/D, D/A and TTL signal transfer. Specially developed software offers the opportunity to control the valves and to monitor the UV and pressure signals, thus permitting complete automatisation. The system was developed for optimum use of the preparative membrane ion exchanger units and allows flow-rates up to 1200 ml/min and a maximum pressure of 1.2 bar.

ml/min) and a change in flow direction, which is very important for reducing the elution volume and also helps to prevent fouling of the membrane. Corresponding controlling software, allowing complete automation of the whole process, has been developed and is currently being tested.

Heparin, a highly sulphated surface mucopolysaccharide (M_r 5000–30 000) shows affinity interactions with many enzymes [6], namely enzymes of the nucleic acid metabolism [6], low- and high-density lipoproteins [7], lysosomal hydrolases [6] and many factors of the blood coagulation cascade [8]. In our case we exploit the particularly high affinity interaction that exists between heparin and ATIII [9] by covalently coupling the former to a membrane. The method of reductive amination [10] was chosen because of its reported ability to couple heparin in an oriented and stable manner [11].

EXPERIMENTAL

Cell culture

Cells. A rat/mouse hybridoma (HB 58; ATCC) and a mouse/mouse hybridoma were cultivated. Both cell lines secreted monoclonal antibodies type IgG$_1$ with an isoelectric point p$I \geqslant 7$. The recombinant Chinese hamster ovary (rCHO) cell line produced human active recombinant antithrombin III (rATIII) [12], a glycoprotein which is an important regulator of blood coagulation.

Medium. A serum-free standard medium [13] containing human transferrin (10 mg/l), bovine insulin (10 mg/l) and bovine albumin (1 g/l) complexed with oleic acid as the main protein components was used. A lipoprotein fraction (Excyte I; Bayer Diagnostic, Munich, Germany) was added (1 ml/l). On the 100-l scale and for the cultivation of the CHO cell line, albumin was omitted.

Bioreactors. The cell lines were cultivated in a 2-l bench-scale perfusion system (Biostat BF 2) and in a 100-l bioreactor (Biostat 100 L) (Braun Biotech International, Melsungen, Germany). pO$_2$ was set at 40% air saturation, pH at 7.1, stirrer speed at 30 rpm and temperature at 37°C.

Membrane ion exchangers

The studies were performend with Sartobind membrane ion exchangers, which were kindly supplied by Sartorius (Göttingen, Germany). A characterization of the membranes is given in Table I.

The *high-flux system* (*HFS*) (Fig. 2) consists of a gear-wheel pump (Watson Marlow 5003 U), a UV monitor (Uvicord; Pharmacia, Uppsala, Sweden), a pressure transmitter (Kistler Instruments, Winterthur, Switzerland) and special valves (Type 700; Bürkert, Ingelfingen, Germany). A personal computer was equipped with interfaces for A/D, D/A and TTL signal transfer. Specially developed software offering the opportunity to control the valves and to monitor the UV and pressure signals, thus allowing complete automation, has been developed and is currently being tested. The system was developed for optimum use of the preparative membrane ion exchanger units and allows flow-rates up to 1200 ml/min and a maximum pressure of 1.2 bar. In order to reduce the elution volume and to prevent fouling, the flow direction was changed between loading and the other steps (Fig. 3).

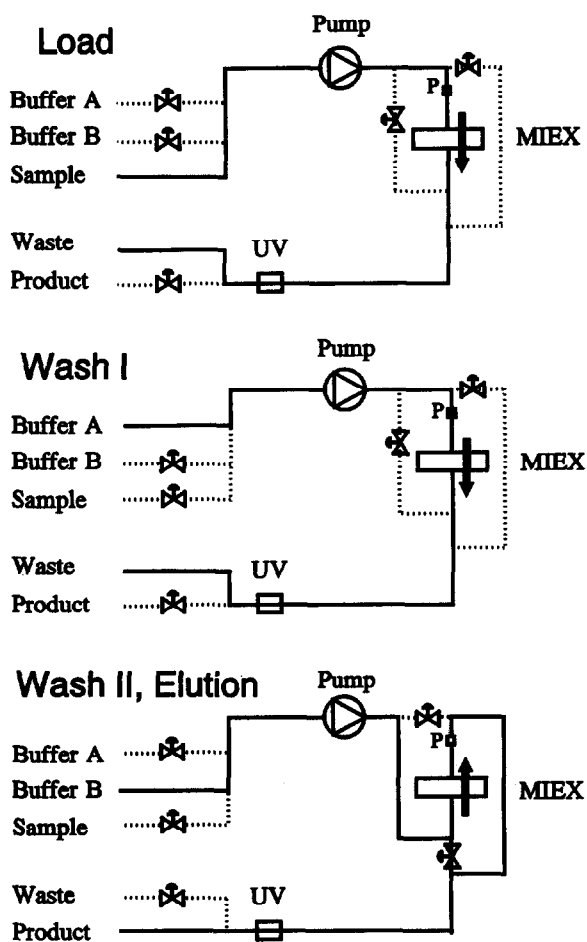*Sample preparation.* In order to bind proteins to the ion exchanger, the conductivity of the

Fig. 3. Process diagram of the HFS. Load: the sample is filtered through the membrane. Wash I: the membrane is washed with buffer A to remove unbound proteins. Wash II: a change in the flow direction (back-flush) of buffer A is used to prevent fouling of the membrane. Elution: buffer B eluates the bound protein.

TABLE I

CHARACTERISTICS OF THE SARTOBIND MEMBRANES S, Q AND A

Characteristic	Value
Membrane material	Synthetic copolymer
Filtration area	12.6, 100, 1400, 2400 cm^2
Type	S = strongly acidic (sulphonic acid);
	Q = strongly basic (quaternary ammonium);
	A = amine modified
Membrane thickness	170–190 μm
Maximum operation pressure	4 bar (400 kPa)
Pore size	0.45 μm
pH stability	pH 2–13
Thermal stability	121°C
Binding capacity	S $\approx$ 1 mg IgG/cm^2;
	Q $\approx$ 0.15 mg ATIII/cm^2;
	A $\approx$ 91 μg ATIII/cm^2

supernatant had to be reduced from 13 mS/cm (normal cell culture medium) to 1.3–1.8 mS/cm. In addition, some of the supernatants were first concentrated by ultrafiltration. To achieve this reduction in conductivity, several methods were used, including a diafiltration cross-flow system (S10Y30, SP20; Amicon, Beverly, MA, USA), an electrodialysis system (StanTech, Geesthacht, Germany) and simple dilution with water (Fig. 4). After adjusting the pH to 5.8 for MAbs and pH 7 for rATIII, the supernatant was passed through the S- and Q-type membrane ion ex-
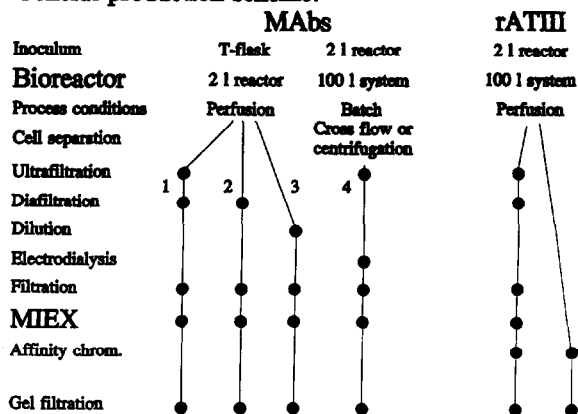
General production scheme:

Fig. 4. Schematic diagram of the general processes used in the production and purification of MAbs and rATIII. The different sample preparation steps for the membrane ion-exchange chromatography are presented together with the purification process employing the amine-modified affinity membranes.

changers, respectively, using the HFS or FPLC system. The product was eluted from the membrane by changing the salt concentration.

Buffers. For the acidic ion exchanger, buffer system I was (A) 25 mM 2-(N-morpholino)ethanesulphonic acid) (MES)–10 mM NaCl (pH 5.8) and (B) 25 mM MES–250 mM NaCl (pH 5.8); buffer system II was (A) 10 mM sodium-phosphate–10 mM NaCl p(H 5.8) and (B) 10 mM sodium-phosphate–250 mM NaCl pH 5.8. For the basic ion exchanger, the buffer system was (A) 20 mM Tris–HCl (pH 7) and (B) 20 mM Tris–HCl–250 mM NaCl (pH 7).

Cleaning and regeneration. Using 0.2 M NaOH (60°C, 20 min), the membranes could be cleaned without loss of binding capacity [14] and were regenerated by washing with buffer A.

Heparin affinity membranes

Material. An LC system (Pharmacia Biosystems) consisting of a fraction collector, a UV monitor, a recorder and a peristaltic pump was used for these studies. The matrix was epoxy- or amine-modified synthetic copolymer membranes (Sartobind; Sartorius).

Coupling procedure with amine-modified membrane. The amine-modified membrane, a single sheet 4.7 cm in diameter, was washed thoroughly with 0.2 M phosphate buffer (pH 7.2), placed in a sealed test-tube containing 20 mg of heparin (sodium salt, from porcine intestinal mucosa, 180 USP units/mg; Sigma, St. Louis, MO, USA) and 15 mg of sodium cyanoborohydride (NaCNBH$_3$) (Fluka, Buchs, Switzerland) in 5 ml of 0.2 M phosphate buffer (pH 7.2) and then allowed to mix end-over-end for 8 days at room temperature. Completion of the coupling was determined by analysing the heparin content of the coupling solution by the method of Dubois *et al.* [15].

The remaining NH$_2$ groups were acetylated according to Baues and Gray [16] with 10 ml of acetic anhydride and 20 ml of 0.2 M sodium acetate, starting at −20°C and mixed end-over-end for 60 min at room temperature. After thorough washing in buffer A the membrane was placed in a membrane holder (Sartorius), which reduced the effective surface area of the membrane to 12.56 cm^2, and equilibrated with 200 ml of buffer A for the first run.

Coupling procedure with epoxy-modified membrane. The 100 cm^2 epoxy-modified membrane module was placed in a coupling array consisting of a peristaltic pump and a reservoir allowing the respective solutions to be circulated through the membrane. The membrane was washed with 300 ml of 0.2 M phosphate buffer (pH 7.2) (without circulation) and then treated for 3 days with 200 ml of 0.2 M ethylendiamine (EDA), with the pH adjusted to 8.5 in order to prevent damage to the casing of the module. The membrane was then washed one way with 500 ml of 0.2 M phosphate buffer (pH 7.2). The coupling solution, consisting of 30 mg of heparin (sodium salt, from ovine intestinal mucosa, 182 IU/mg; Sigma) and 30 mg of NaCNBH$_3$ in 9 ml of 0.2 M phosphate buffer (pH 7.2) was circulated over the membrane for 5 days. After 3 days an additional 50 mg of NaCNBH$_3$ in 1 ml of 0.2 M phosphate buffer (pH 7.2) were added. The remaining NH$_2$ groups were again removed by acetylation.

Sample run. A typical sample run consisted of equilibration with 100 ml of buffer A (10 ml/min) followed by loading of the membrane with supernatant, pH adjustment to 5.8 (1–10 ml/min), a wash step with 100 ml of buffer A and finally elution with buffer B (both 10 ml/min).

Buffers. The buffers were (A) 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES)–150 mM NaCl (pH 5.8) and (B) 20 mM HEPES–2.5 M NaCl (pH 7).

Cleaning. In order to prevent fouling of the membrane equilibration, washing and elution were always performed in a different flow direction to loading, resulting in backflushing of the system. Additional cleaning of the membrane with 2.5% sodium dodecyl sulphate (SDS) solution was also successful and resulted in removal of clogging material from the membrane, so that the original flow-rates were restored.

Final purification. This was carried out using a preparative gel filtration column (Superdex 200 PG; Pharmacia) with a 600-ml gel volume. A 20 mM phosphate buffer containing 150 mM NaCl was used. The flow-rate was set at 6 ml/min.

Analytical methods

Antibody concentrations were determined using a standard kinetic sandwich enzyme-linked immunosorbent assay (ELISA) method or an ABICAP affinity column (Abion, Jülich, Germany) for rapid analysis. rATIII concentrations were also determind by kinetic ELISA (Behring Werke, Marburg, Germany). Purity was determined by SDS–polyacrylamide gel electrophoresis (PAGE) [17] either automatically (Phast System; Pharmacia) using silver staining [18] or manually (Multiphor II; Pharmacia) using Sensi-quant Coomassie Brilliant Blue staining [19]. The silver-stained gels were scanned using the Gel-Image 1DEVA software (Pharmacia).

The concentration of heparin in the coupling solution was determined using the phenol-based method described by Dubois *et al.* [15].

Conversion factor. In order to compare membraneous and gel matrices with respect to ligands and protein bound, a conversion factor, obtained from Sartorius [20], equalling 50 cm^2 of membrane surface with 1 ml of gel matrix was used. It is also important to note that the 17.35-cm^2 membrane was placed in a filter holder that reduced the effective surface area to 12.56 cm^2. All calculations made refer to these values unless stated otherwise.

RESULTS AND DISCUSSION

Purification of MAbs with membrane ion exchangers 100-cm^2 membrane ion exchanger

In order to find the optimum conditions for protein binding to the membrane ion exchanger, supernatant feed solutions of various pH and ionic strength were applied to a 100-cm^2 module (Fig. 5). It was found that a pH of 5.8 and a corresponding ionic strength of 1.8 mS/cm^2 yielded the best results (96% recovery). During the runs conducted so far over 50 mg of IgG per module could be bound employing a maximum flow-rate of 30 ml/min. The concentration factor was about 50-fold, which is comparable to that of other ion-exchange matrices. Most of the albumin could be separated by gradient elution; human transferrin, however, was concentrated under the conditions stated above (Fig. 6).

Preparative purification of MAbs using the HFS

The capacity of the membrane ion exchanger of 1 mg IgG/cm^2 (equivalent to 50 mg IgG/ml bed volume), as determined with a single filter
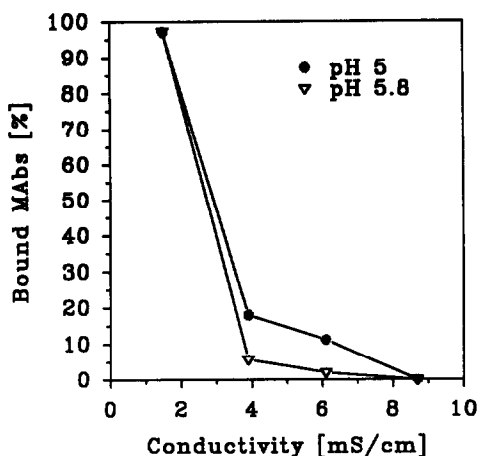

Fig. 5. Relationship between conductivity of the supernatant and binding of MAbs using the 100-cm^2 membrane ion exchanger with a flow-rate of 10 ml/min. In each run 7 mg of MAbs were applied to the membrane. In order to bind more than 90% it was necessary to reduce the conductivity to values below 1.8 mS/cm. Buffer systems: ● = pH 5, 25 mM acetic acid–10 mM NaCl; ∇ = pH 5.8, 25 mM MES–10 mM NaCl; the MAbs were eluted with the corresponding buffer with 250 mM NaCl.
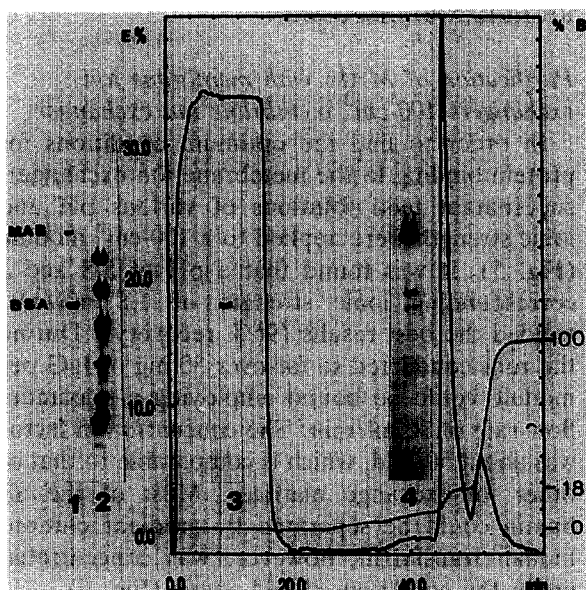
Fig. 6. Purification of monoclonal antibodies from cell culture supernatant. Following the diafiltration step the supernatant (9 mg of MAbs) was applied to the membrane ion exchanger at a flow-rate of 6 ml/min [buffer system I, MES (pH 5.8)]. The binding rate was 95%. Using a gradient from 0 to 8% B for 20 min, impurities were separated. With an 18% step of buffer B, 90% of the MAbs could be eluted. The membrane was flushed with 100% B and washed with buffer A. SDS-PAGE (unreduced) shows the purification process. Samples were diluted 1:1 with buffer. Lanes: 1 = crude cell culture supernatant (150 ml); 2 = low-molecular-mass marker (reduced); 3 = breakthrough (180 ml); 4 = MAbs eluted with 18% B (23 ml); the last peak contains unknown components but no proteins.

Fig. 7. SDS-polyacrylamide gradient gel (8–25%) using a modified silver stain under unreduced conditions. Isolation of MAbs with strongly acidic membrane ion exchanger and gel filtration. Lanes: 1 = marker, M_r 14 000–96 000 (reduced); 2 = supernatant; 3 = supernatant after salt reduction; 4 = unbound proteins (MIEX); 5 = first wash (MIEX); 6 = concentrated IgG (MIEX); 7 = gel filtration 1 peak; 8 = gel filtration 2 peak, purified MAbs. kD = kilodalton.

sheet (12.56 cm^2 effective surface), is very high compared with filter materials from other suppliers. The preparative units could bind up to 910 mg without any decrease in recovery (larger amounts were not determined). Lowering the ionic strength of the supernatant by method I, II or IV (Fig. 4) resulted in binding rates of about 95% and recovery rates in excess of 80% (Table II). The recovery was found to be independent of the concentration of the supernatant. The only exception occurs if the relationship between the concentration of the MAbs and the volume in which they are dissolved becomes too unfavourable. A decrease in the ionic strength by simple 1:8 dilution with water (III) resulted in lower binding capacities if the feed solution exceeded a certain volume (>6 l), but in this
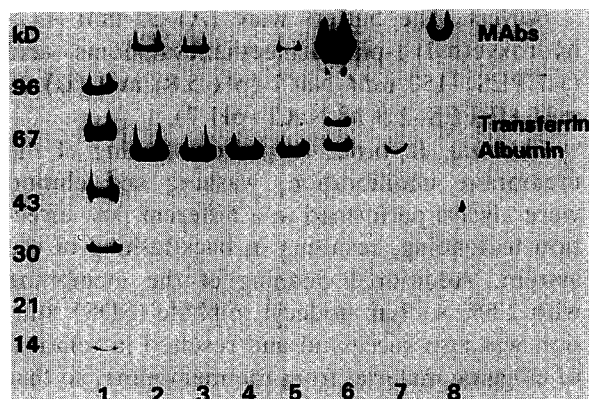
instance no expensive membrane step such as diafiltration was necessary. The concentration factor achieved by the MIEX is still unsatisfactory in comparison with a common ion exchanger (Table III), but optimization of the ion exchanger unit towards smaller elution volumes could change that. Up to a flow-rate of 571 l/m^2 · h (maximum achieved by the system) the binding of the MAbs on the MIEX was better than 90%. The concentration of 5 g of IgG in several cycles with one unit was possible; sub-
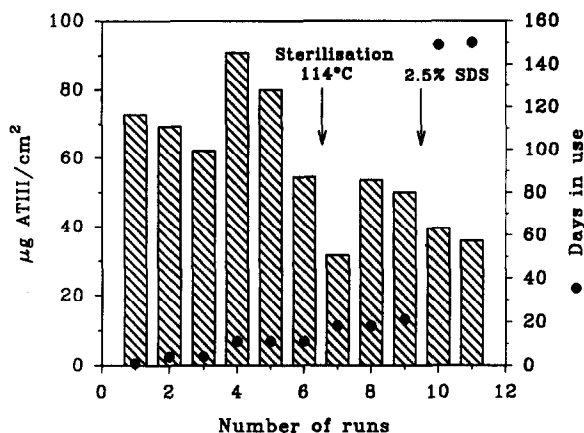

Fig. 8. Repeated use of the amine-modified membrane over a period of 150 days. Effects of steam sterilization and treatment with 2.5% SDS.

TABLE II

SUMMARY OF DIFFERENT SAMPLE PREPARATION STEPS AND THE SUBSEQUENT MEMBRANE ION-EXCHANGER RUNS

Within minutes gram amounts of product could be purified in the HFS [buffer system I, sodium phosphate (pH 5.8)]. The concentration factor was calculated by dividing the IgG concentration in the elution by the IgG concentration in the supernatant. The recovery was found to be independent of the concentration of the supernatant. Lowering the ionic strength of the supernatant by method I, II or IV resulted in binding rates of about 95% and recoveries in excess of 80%.

Method	No.	IgG (mg)	Volume (ml)	IgG bound (%)	Flow-rate (ml/min)	Recovery (%)	Concentration factor	Process time (h)	Throughput (g/h)	IgG species
(I) Ultrafiltration and	1	320	2010	99	750	81	13.6	0.08	4.0	Rat
diafiltration	2	187	5200	95	866	93	10.7	0.14	1.3	Mouse
	3	319	5500	99	845	81	12.7	0.15	2.1	Mouse
	4	910	1000	97	843	84	1.8	0.06	14.3	Mouse
	5	910	1000	99	843	94	2.6	0.06	14.3	Mouse
(II) Diafiltration only	6	187	5200	95	866	93	10.7	0.14	1.3	Mouse
	7	166	4600	99	836	96	14.3	0.13	1.3	Mouse
	8	43	3300	95	843	81	8.9	0.11	0.4	Mouse
(III) Dilution of the	9	146	3500	91	845	91	10.3	0.11	1.3	Mouse
supernatant with	10	139	5600	94	845	91	17.7	0.15	0.9	Mouse
water	11	37	16700	73	843	68	17.2	0.37	0.1	Rat
	12	110	14300	68	843	66	22.1	0.32	0.3	Rat
	13	240	34500	63	843	57	122	0.72	0.3	Rat
(IV) Ultrafiltration and	14	456	1400	91	845	90	3.1	0.11	4.2	Mouse
electrodialysis	15	489	1500	91	845	84	4.0	0.11	4.3	Mouse
	16	391	1200	98	845	75	3.0	0.11	3.6	Mouse
	17	267	1100	97	1060	80	4.4	0.08	3.3	Mouse

TABLE III

COMPARISON BETWEEN A GEL-MATRIX CATION EXCHANGER (S-SEPHAROSE FF) AND THE STRONGLY ACIDIC MEMBRANE ION EXCHANGER (SARTOBIND S)

Parameter	Matrix	
	S-Sepharose FF	Sartobind S
Bed volume/area	28 ml (XK 50 × 30)[a]	1400 cm^2
Supernatant volume (ml)	4700	5500
Flow-rate (ml/min)	80	845
MAbs applied (mg)	359	319
MAbs bound (%)	69	99
Recovery (%)	67	81
Concentration factor	58	13
Process time (h)	1.50	0.15
Throughput (g/h)	0.16	2.10

[a] Pharmacia, S.

sequently a treatment with 0.2 M NaOH (60°C, 20 min) restored the flow. So far one MIEX unit has been used 25 times without decreases in binding capacity, recovery or flow-rate. The main advantage of the MIEX in comparison with an ion exchanger based on gel matrices is the much higher throughput (>13-fold) (Table III). Final purification of the MAbs was carried out using a preparative gel filtration column. The elutions from the MIEX were applied directly, without prior concentration, to the gel filtration column and resulted in two distinct peaks. The second peak contained the purified MAbs (Fig. 7).

Purification of rATIII

Affinity membranes. The 17.35-cm^2 amine-modified membrane covalently coupled 5.2 mg of heparin (porcine), corresponding to *ca.* 0.3 mg heparin/cm^2 matrix, which is slightly higher

than that obtained by Sasaki et al. (0.2 mg/cm^2) [11]. The coupling took fairly long to complete (8 days), but this is not uncommon for reductive amination [10,21] and the optimized conditions that were taken from the paper by Sasaki et al. [11], which were obtained using amino-Sepharose as a matrix, might not apply completely to the membraneous matrix used in this instance. The maximum amount of rATIII that could be adsorbed to the amine-modified membrane was 1.14 mg (Fig. 8), which amounts to a binding capacity of 90.8 μg/cm^2 (effective surface area 12.56 cm^2). This value equals or exceeds that of other commercially available heparin affinity matrices. The stability achieved by the method of reductive amination used in this instance was very satisfactory especially when compared with the known shortcomings [10] of products coupled by the cyanogen bromide method. The amine-based heparin-affinity membrane was used eleven times over a period of about 5 months, purifying a total of 8.2 mg of rATIII. It was also sterilized by steam sterilization (15 min at 114°C) after six runs, losing about 38% of its capacity (average of the first six runs, 71.7 μg rATIII/ cm^2; average of the last five runs, 43.9 μg/cm^2) and treated sucessfully at one time with 2.5% SDS solution to remove clogging material from the membrane surface.

The 100-cm^2 module containing an epoxy-activated membrane, which was first treated with ethylenediamine in order to produce sufficient spacers, coupled 12 mg of heparin (ovine) in 5 days, which corresponds to 0.18 mg heparin/ cm^2. The best run resulted in the purification of 3.95 mg of rATIII, which denotes a binding capacity of 39.5 μg/cm^2. The 100-cm^2 module purified a total of 18 mg of rATIII in eight runs over a period of almost 3 months (Fig. 9). The 100-cm^2 module lost considerable binding capacity (about 65% when comparing the average of the first three runs with that of the last five) (Fig. 9). This loss might be due to storing the filter without first removing the clogging material. Treatment with 2.5% SDS after the fifth run, however, resulted in an improvement of the rATIII binding capacity (Fig. 9).

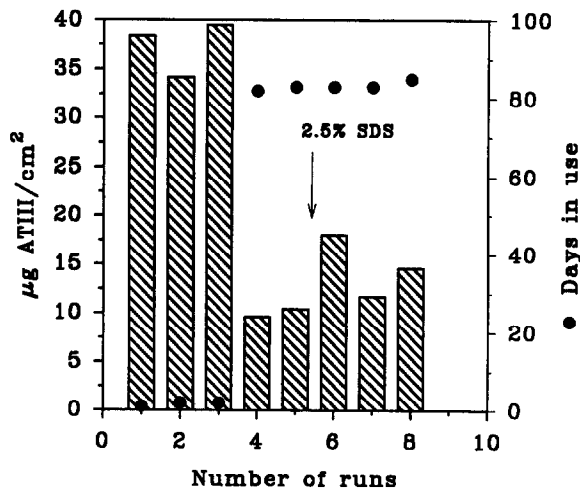The initial purity of the eluates was about 75%

Fig. 9. Repeated use of the epoxy-modified membrane over a period of 85 days. Effect of treatment the membrane with 2.5% SDS.

(as determined with the Gel-Image system), with impurities mainly in the lower-molecular-mass range. These non-specific bindings might be due to the many affinity interactions of heparin [6], or the fact that heparin as a highly sulphated saccharide polyanion has some of the properties of a cation exchanger. The low-molecular-mass protein impurities were readily removed by gel filtration (Fig. 10).
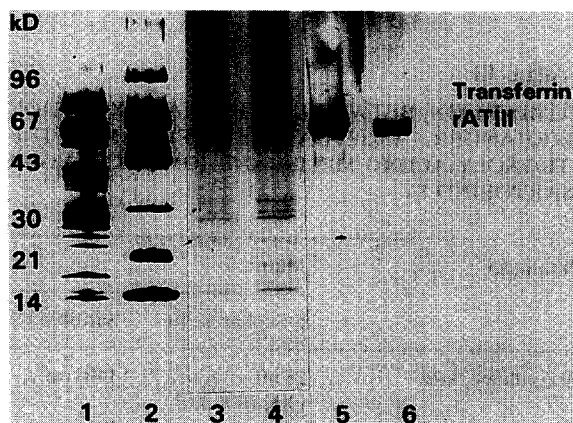

Fig. 10. SDS polyacrylamide gradient gel (8–25%) using a modified silver stain under unreduced conditions. Isolation of rATIII with basic preparative ion exchanger, amine-modified affinity membranes and gel filtration. Lanes: 1 = supernatant after ultra- and diafiltration (concentrated tenfold); 2 = marker, M_r 14 000–96 000 (reduced); 3 + 4 = elution of rATIII from MIEX Q; 5 = elution of rATIII from affinity membrane; 6 = pure rATIII (after affinity membrane and subsequent gel filtration.)

TABLE IV

CONCENTRATION OF rATIII WITH THE STRONGLY BASIC MEMBRANE ION EXCHANGER (2400 cm^2) USING THE HFS

No.	rATIII (mg)	Volume (ml)	rATIII bound (%)	Flow-rate (ml/min)	Recovery (%)	Concentration factor	Process time (h)	Throughput (g/h)
1	210	2000	100	560	99	3.9	0.08	2.6
2	240	2000	100	560	99	2.5	0.15	1.5
3	140	550	98	380	98	1.6	0.51	0.3
4	344	7500	100	580	96	14.4	0.51	0.6
5	366	8000	100	600	79	11.8	0.47	0.8
6	113	5000	93	610	79	13.1	0.31	0.5
7	110	3500	85	620	82	11.9	0.22	0.5
8	68	4500	100	620	98	22.0	0.28	0.3
9	18	3500	100	620	92	17.8	0.36	0.05

The affinity of the heparin membranes towards human transferrin, the most critical contaminant in this instance, is very weak. Ionic interactions with the matrix were further diminished by adjusting the pH of the supernatant to 5.8, the isoelectric point of human transferrin.

Preparative purification of rATIII using the HFS. The 2400-cm^2 units could be run with flow-rates up to 620 ml/min, achieving binding rates of 97% and recoveries of about 91% (Table

TABLE V

COMPARISON OF A GEL-MATRIX ANION EXCHANGER (Q-SEPHAROSE FF) AND THE STRONGLY BASIC MEMBRANE ION EXCHANGER (SARTOBIND Q)

Parameter	Matrix	
	Q-Sepharose FF	Sartobind Q
Bed volume/area	30 ml (XK 50 × 30)[a]	2400 cm^2
Supernatant volume (ml)	4200	7500
Flow-rate (ml/min)	60	580
rATIII applied (mg)	246	344
rATIII bound (%)	100	100
Recovery (%)	95	96
Concentration factor	42	14
Process time (h)	1.70	0.51
Throughput (g/h)	0.15	0.60

[a] Pharmacia, S.

IV). The estimated capacity of the anion exchanger membrane for rATIII of 0.15 mg/cm^2 (360 mg/unit) is low compared with the capacity of the S type for MAbs; this might be due to the competive binding of DNA and other negatively charged biomolecules under these conditions. The purity of the product was 75%, with human transferrin as one of the main impurities (Fig. 10). In comparison with standard ion exchangers (Table V), MIEX shows similar binding and recovery rates. Only the concentration factor (elution volume) could be improved in the future. Purification with MIEX resulted in a throughput more than four times higher than Q-Sepharose FF.

CONCLUSIONS

Membrane ion exchanger and affinity membranes are versatile tools for the concentration and purification of MAbs and rATIII. The binding capacity, the recovery and the concentration factor (only by the 100-cm^2 unit) are similar to those for gel matrices. The main advantage is the very high throughput. They are easy to handle and can be used repeatedly.

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