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Large-scale membrane adsorbers

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Abstract

A new large-scale membrane adsorber system for rapid separation of biomolecules based on a hollow cylindrical module geometry in unitized construction and combined parallel and serial connection of modules is presented. The effectiveness of the concept is demonstrated by breakthrough curves on a 21-m^2 pilot plant with hemoglobin as a model substance. Scale-up to the order of 100 m^2 and above is easily performed by combining modules in parallel for increasing capacity, and in series for optimizing performance. An estimation of productivity based on these data is presented. Preliminary results of separations by linear salt gradient elution are also given.

Keywords: Membranes; Instrumentation; Adsorbers; Proteins

1. Introduction

Adsorptive membranes are increasingly used for the isolation and purification of biomolecules, a recent review contains 104 references [1]. Sartobind membranes (Sartorius), which are flat sheet membranes with a methacrylic polymer grafted onto a microporous support and bearing ionic groups, have found numerous applications at different laboratories [2–13]. For the first generation of this product line [14,15] the support was a 0.45-μm nominal pore size nylon membrane, which was later substituted by a crosslinked regenerated cellulose membrane of 3 μm nominal pore size with a high stability against chemical and enzymatic attack and an extremely low nonspecific adsorption [16,17]. This second generation of Sartobind membranes was first used for circular, stacked membrane adsorber modules of up to 0.5 m^2 membrane area [18] which were successfully used for semitechnical separations [19]. For a further scale up a cylindrical module geometry proved to be superior. Based on this concept a unitized system of modules and hardware was developed, which makes the design of large scale plants with membrane areas up to 100 m^2 and more, feasible [20]. The present contribution, which is a revised and extended version of a previous presentation [21], is divided into two parts: in Part I the Sartobind Large Scale System is described and in Part II the first experimental results with this technology are presented.

2. Part I: Sartobind large scale system

The main feature of the Sartobind large scale system is a unitized construction to attain high versatility with as few different component as possible. The system also includes a series for small scale applications, which is intended for preliminary trials. Central parts of the whole system are the membrane adsorber modules, the housings wherein they are to be operated and technical guidelines for

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the design of large plants by a combination of parallel and serial connections of modules.

2.1. Sartobind cylindrical modules

A detailed description of the system can be found on the website http://www.sartorius.com.

In principle a Sartobind large scale membrane adsorber module consists of the membrane, reeled to form a hollow cylinder, with screens of stainless steel on the inner and outer sides, which are embedded in plastic caps on both ends.

The length of the Sartobind standard modules is 50 cm (20 in.). They are available in configurations with nominal 15, 30 and 60 membrane layers corresponding to nominal membrane areas of 2, 4 and 8 m^2, respectively. They all have the same outer diameter (96 mm) and fit into the same housings. There are three different cores available accommodating the different module geometry. The inner and outer channel volumes are equal and add up to 600 ml of total channel volume.

For geometric reasons doubling the membrane area does not exactly correspond to a doubling of the number of the layers. Furthermore the thickness of the membrane varies to a certain extent. So the different configurations are more strictly characterized by the membrane volumes.

The fluxes of the different configurations do not correspond directly to the number of layers. The absolute values of the fluxes are highly dependent on the medium and the type of membrane. The order of magnitude for the flux with 10 m*M* potassium phosphate buffer, pH 7.0 is referred to as 'nominal flux'. An overview of these data is given in Table 1.

Sartobind modules are also available in lengths of 25, 12.5, 6.2 and 3.1 cm, the smallest unit representing 1/16 of a standard module.

2.2. Parallel connection

Several Sartobind standard modules of the same geometry can be operated in parallel connection in a common housing as a stack. For the upper modules elongation cores are available, which connects the inner channels of the modules (Fig. 1, first stage).

The number of modules which can be connected in this way without loss in performance is limited by the pressure drop in the channels, which can result in an uneven distribution of differential pressures along the modules and deteriorate the break through behavior. It can be estimated that this effect may become significant with more than 2, 3 and 5 standard modules of 15, 30 and 60 layers, respectively, in a common housing. Details of the calculation, which is based on the assumption of laminar flow in the channels, are given in [22]. Considering the Reynolds number it can be estimated, that this is valid, in the same order, at least up to flow-rates of 18.5, 16.4 and 11.3 l/min, respectively.

If higher flow-rates per housing are to be expected, a further increase of membrane area is obtained by parallel connection of two or four housings equipped with an equal number of modules. Symmetrical manifolds and stands for a stage of four housings are part of the system. A stage of 96 m^2 nominal membrane area for example, consisting of 12 modules of 8 m^2 in four housings, has only an area of 50×50 cm and a total height of about 2 m.

2.3. Connection in series

Any parallel connection of adsorber modules

Table 1
Data of Sartobind 50 cm (20 in.) standard modules

Code	Nominal membrane area (m^2)	Nominal number of layers	Membrane volume (l)	Nominal flux (l/min ×100 KPa)
MA-20K-15-50[a]	2	15	0.55	15
MA-40K-30-50	4	30	1.1	7.0
MA-80K-60-50	8	60	2.0	2.5

[a] Code number characterizes membrane chemistry (MA is to be substituted by S for sulfonic acid or by Q for quaternary ammonium groups), the membrane area (e.g. 20K for 20 000 cm^2), followed by the number of layers and the length in centimeters.

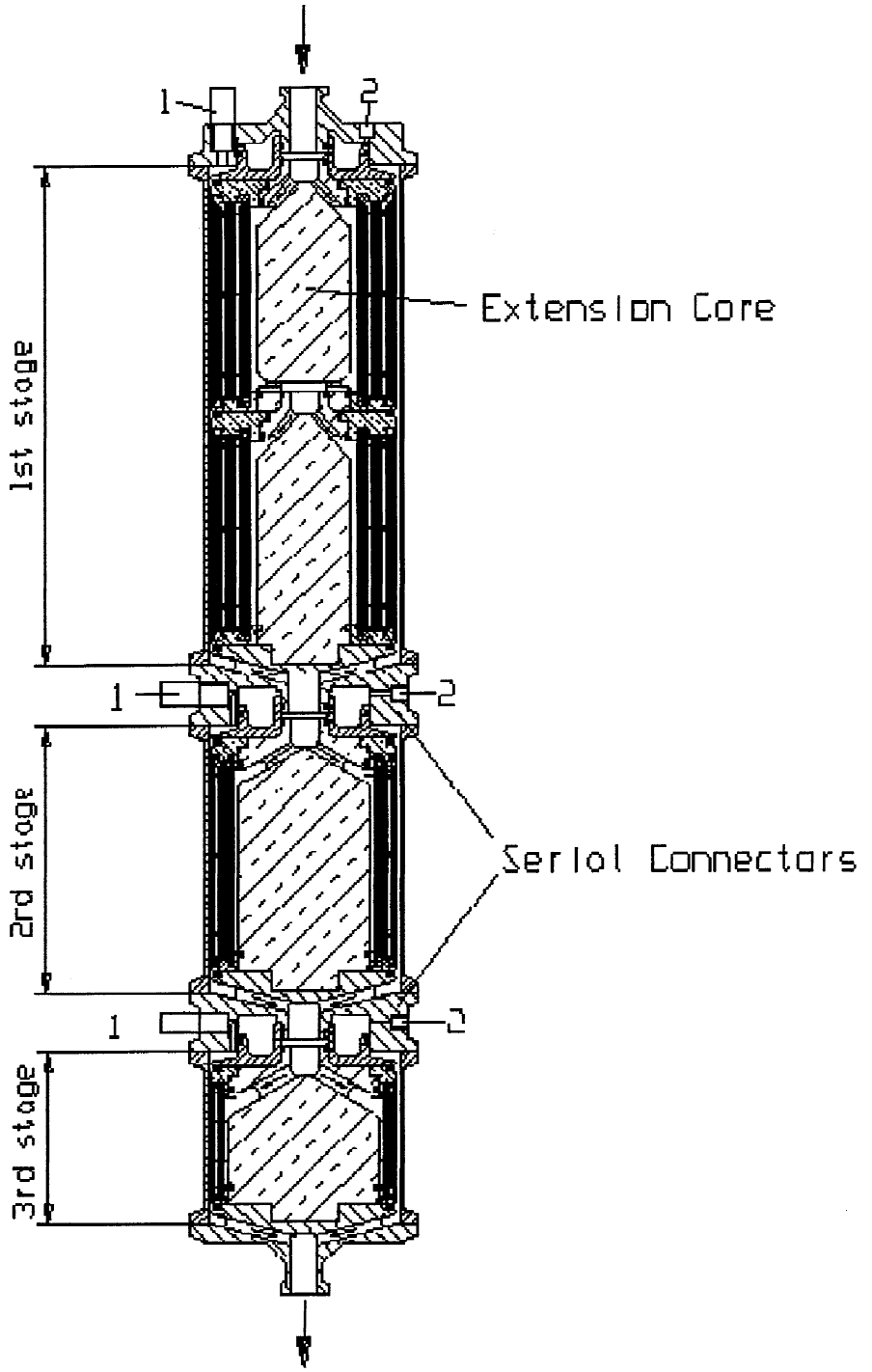

Fig. 1. Cross section of 21 m^2 3-stage Sartobind pilot plant. Not true to scale (reduced in length to 1/5). (1) Venting valves; (2) pressurized air connectors for the actuation of the pressure plates. All components are made from AISI 316L (1.4435) stainless steel, the pressure plate is made from polyoxymethylene (POM).

Table 2
Module equipment (sulfonic acid type) of the Sartobind pilot plant used for breakthrough curve experiments

Stage	Module type	Number of modules in Fig. 2	Membrane area (m^2)	% of total area
1	S-80K-60-50	146+147	16	76
2	S-40K-30-50	150	4	19
3	S-10K-15-25	152	1	5.0

reduces flow resistance, but the performance deteriorates because inequalities in flux or capacity among the modules cause a loss of dynamic capacity of the whole system. On the other hand, connection in series improves breakthrough behavior, but increases flow resistance. Therefore, optimal results are to be expected by a combination of parallel and serial connections in a multistage plant.

It is obvious that the optimal utilization of the total installed membrane area is achieved when the capacity of all stages is exhausted simultaneously. This can be attained by installing decreasing membrane areas in series in different stages. If, for example, a 84 m^2 3-stage plant consists of 8 modules with 60 layers in the first, 4 modules with 30 layers in the second and 2 modules with 15 layers in the third stage, all the stages exhibit similar pressure drops and any stage has 25% of the static capacity of the preceding one.

For small multistage plants a serial connector is available, which can be mounted between the housings and leads the flow through from the outer channel of the preceding (upper) stage to the inner channel of the following one. A 21 m^2 3-stage pilot plant based on this concept was assembled in this way (Fig. 1) and has been operated successfully (see Part II). The module equipment is listed in Table 2.

3. Part II: Experimental results

Since it proved difficult to find a source for large quantities of a pure protein it was decided to use hemoglobin freshly prepared from bovine blood, because commercially available hemoglobin preparations are not completely soluble.

3.1. Materials and methods

All chemicals were of analytical grade (Merck, Darmstadt, Germany), deonized water with a conductivity below 1.5 μS/cm was used throughout. Hemoglobin concentrations were determined using the reagent according to Drabkin against a Hb-standard (both Sigma, Deisenhofen, Germany). Hen egg white lysozyme (HEWL) was a commercial product (Sigma).

For microfiltration and microdiafiltration 2.8 m^2 0.2-μm Hydrosart cross flow cassettes, for ultrafiltration and ultradiafiltration 2.8 m^2 Hydrosart UF cassettes with an M_r 30 000 nominal cut off, both Sartorius (Göttingen, Germany) were used. Liquids were pumped by rotating lobe pumps (Model Labtop 250, Flowtech, Atlanta, GA, USA).

The Sartobind pilot plant was equipped with pneumatically actuated diaphragm valves supplied by the MarcValve (Tewksbury, MA, USA). Control of valves and pumps was performed by a computer program.

Protein was detected in the effluents at 280 nm with an inline photometer model AF44 with 2-mm path length and model 662 UV analyzer (Wedgewood, San Carlos, CA, USA).

3.2. Preparation of crude hemoglobin solution from bovine whole blood

To 4 l of a solution of 3.8% trisodium citrate in 0.9% sodium chloride (isoCl) with 0.02% sodium azide 16 l of freshly withdrawn bovine blood was added and gently mixed. This suspension was added to 140 l isoCl precooled to 6°C and mixed. The temperature was maintained at 6°C by means of an immersed cooling coil. The blood cell suspension was cross flow microdiafiltrated at low speed and minimum pressure under the addition of 150 l isoCl with 0.02% sodium azide. The permeate containing the major part of the plasma proteins was discarded. The remaining suspension was concentrated to 40 l by microfiltration. The hemoglobin loss due to hemolysis was below 1%.

The erythrocytes then were lysed by the addition of 60 l water with 0.02% sodium azide, precooled to 6°C. The solution was freed from cell debris by microfiltration and concentrated to a volume of 10 l by ultrafiltration.

The concentrate was allowed to warm to room temperature, adjusted to pH 6.0 with phosphoric acid and diluted with water to a conductivity of 1.0 mS/cm, i.e. an ionic strength equal to 5 m*M* potassium phosphate buffer, pH 6.0. The solution was again microfiltrated to remove any turbidity and checked for pH. The resultant crude Hb solution was adjusted with 5 m*M* potassium phosphate buffer, pH 6.0, to a Hb concentration of 3 g/l.

3.3. Purification of Hb with Sartobind membrane ion exchangers

The objective of the purification step was the removal of residual serum proteins from the crude Hb solution in order to get neat baselines when measuring breakthrough curves. It was performed by ion exchange using the first stage (16 m^2 membrane area) of the Sartobind pilot plant.

Approximately 20 l crude Hb solution representing 60 g Hb were loaded onto the adsorber at a flow-rate of 4.5 l/min at a differential pressure of 130 kPa (19.5 p.s.i.) and washed with 5 l of 5 m*M* potassium phosphate buffer. The Hb was then eluted with 100 m*M* potassium phosphate buffer, pH 6.0, and the main fraction of 4 l collected, which contained about 90% of the Hb used for loading. The location of the main fraction had been determined in a previous trial. After washing the system with 5 l of 5 m*M* potassium phosphate buffer the next run was performed. Loading, rinsing and elution were controlled by a computer program. The total cycle time of the ion-exchange step was ≈7 min.

The pooled eluates of several runs were ultradiafiltrated by the addition of 5 m*M* potassium phosphate buffer, pH 6. The purified Hb was then concentrated to 100 g/l by ultrafiltration and stored at 4°C as a stock solution for the following experiments.

The overall protein recovery was 60% based on the Hb content of the starting material, according to about 1 kg purified Hb from 16 l of blood. The main losses occurred with the slurry remaining in the cassettes after the filtration and concentration steps. No attempt was made to further optimize recovery because of the low value of the starting material.

3.4. Breakthrough curves

Breakthrough curves were measured at the individual modules (Fig. 2) in separate housings. The flow-rate was 1 l/min for the smaller modules Nos. 152 and 150 and 3.5 l/min for the two larger modules Nos. 146 and 147, respectively. Furthermore, the breakthrough was determined at the 3-stage pilot plant which is shown in Fig. 2 and the module set which is given in Table 2 as well as with only the first stage. The feed solution was prepared by dilution of a stock solution of purified hemoglobin by the addition of 5 m*M* phosphate buffer, pH 6.0 to the concentrations indicated in the legends to the figures. The flow-rate was 3.5 l/min at a differential pressure of 54 kPa (8 p.s.i.) at ambient temperature. The resulting breakthrough curves of the first stage (modules No. 146 and No. 147 in parallel connection) and 3-stage plant are shown in Fig. 3A and B, respectively.

3.5. Linear salt gradient elution

Since HEWL is commercially available in large quantities of defined purity, it was chosen as one component of a protein mixture for gradient separation trials.

A 1-g amount of HEWL was dissolved in 2 l of 5 m*M* sodium phosphate buffer, pH 6.0, and 10 ml of a 100 g/l stock solution of purified bovine hemoglobin (1 g Hb) was added. The solution was applied to 3 Sartobind modules of equal nominal membrane areas and different configurations as indicated in Table 3. The flow-rate was 1 l/min.

After rinsing with buffer, a linear gradient was applied at a flow-rate of 1 l/min. The effluent volume was monitored by a balance. The gradient was generated according to the principle of communicating tubes. Two 10-l flasks with an outlet at the bottom were connected with tubing. They were filled with 10 m*M* sodium phosphate buffer, pH 6.0 (starting buffer) and 0.5 *M* sodium chloride in starting buffer (modifier), respectively. As the start-

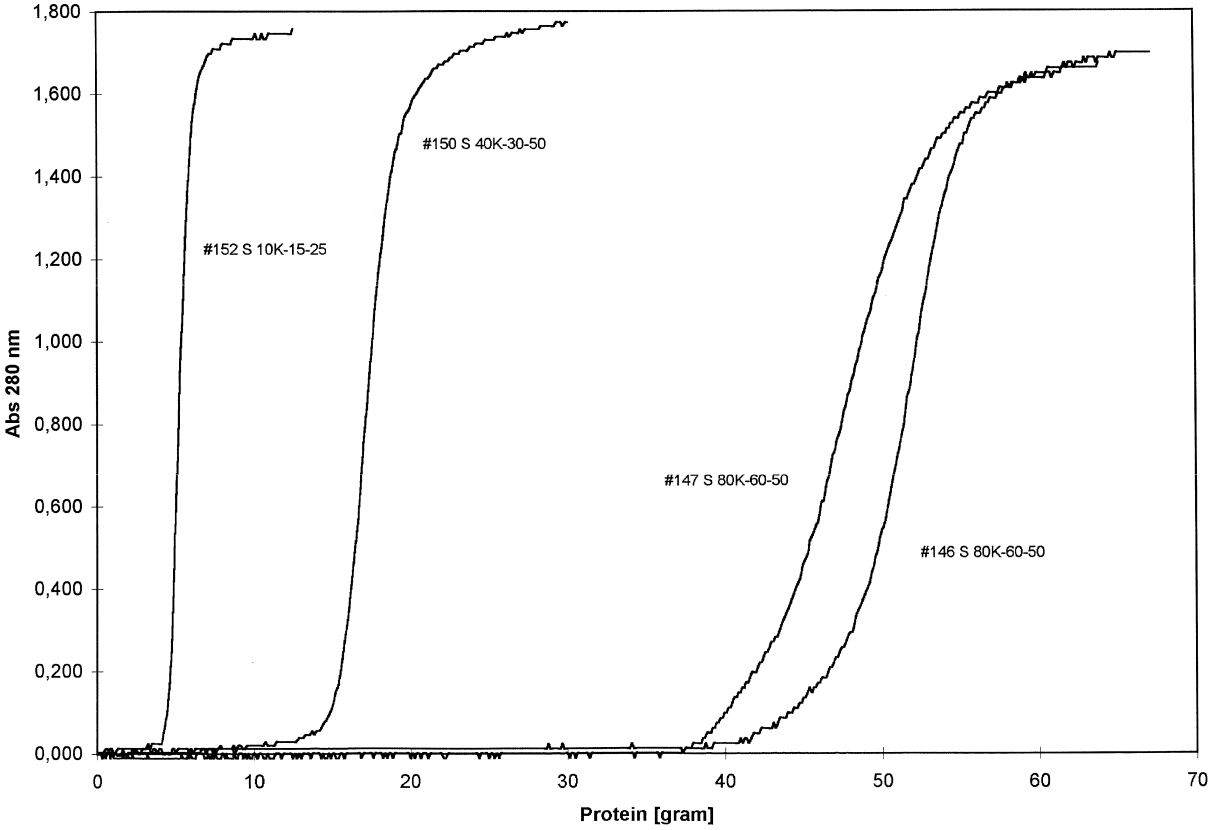

Fig. 2. Breakthrough curves for the individual modules used in the 3-stage pilot plant shown in Fig. 1. The concentration of hemoglobin in the feed was 1.0 g/l.

ing buffer is withdrawn, the modifier is continuously added by thorough mixing and fed to the system.

4. Results

4.1. Purification of Hb with Sartobind membrane ion exchangers

Although originally intended just for preparing sufficient protein for the breakthrough curve trials, the performance of the single stage plant used for the purification step of Hb allows some rough estimation of the productivity of the process on a 16-m^2 plant.

A 60-g amount of Hb per cycle and a cycle time of 7 min corresponds to about 500 g Hb/h or 10 kg per day (20 h) and $2 \cdot 10^3$ kg per year (200 days). This means a productivity of 125 kg/m^2 year — supposing the long term behavior allows such extrapolations, which has to be investigated. Experience with a single module of the S type ranges to about 500 cycles with the present Hb system without any significant change of performance.

4.2. Breakthrough curves

The curves for the individual modules in Fig. 2 show a rather steep breakthrough of protein for the two smaller ones and flatter ones for the two larger ones. This is to be expected because the smaller modules adsorb less protein. The dynamic capacity at 5% breakthrough for the individual four modules amounts to roughly 100 g of Hb.

Comparison of the breakthrough curves of the first

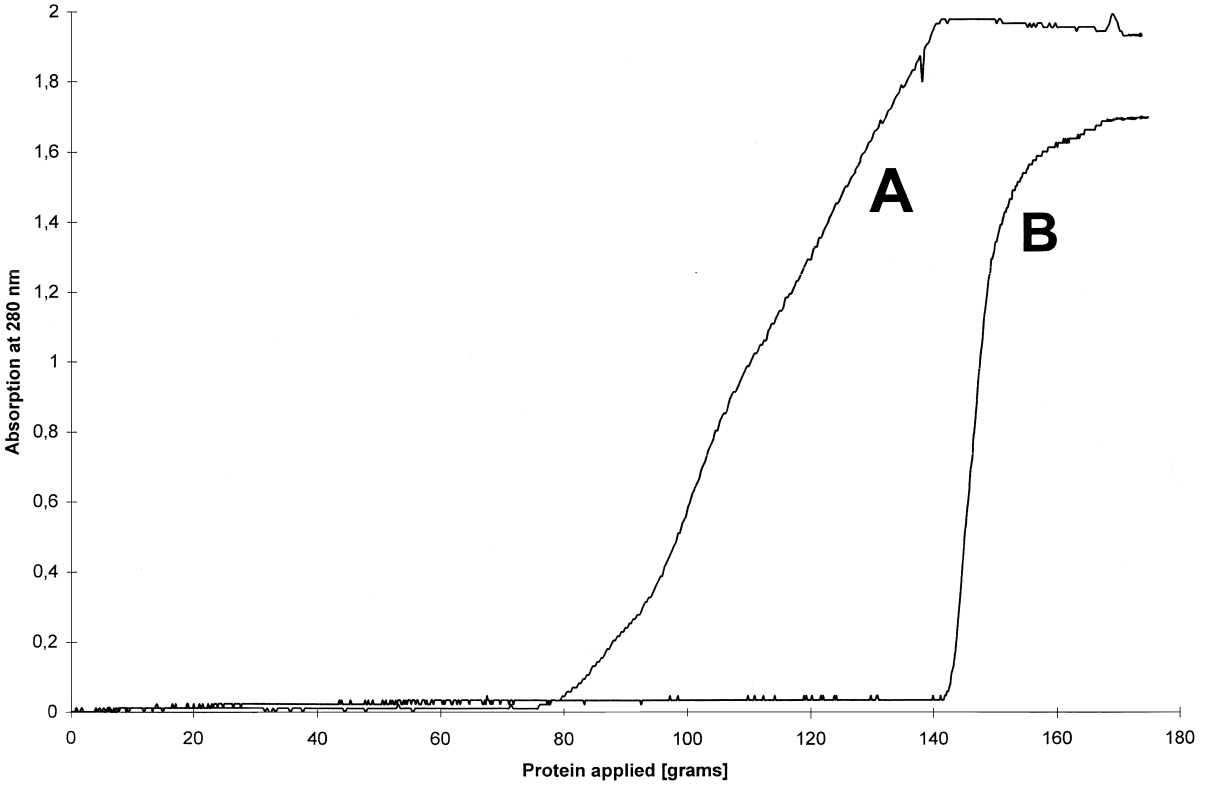

Fig. 3. Effect of multistage connection of adsorbers on break through curves, determined with bovine hemoglobin. (A) Breakthrough curve of the first stage (16 m^2 membrane area).The concentration of hemoglobin in the feed was 1.0 g/l. (B) Breakthrough curve of 3-stage pilot plant (16–4–1 m^2 membrane areas).The concentration of hemoglobin in the feed was 0.86 g/l.

stage alone (Fig. 3A) with that of the complete 3-stage plant (Fig. 3B) shows that a only moderate increase in membrane area (from 16 to 21 m^2, i.e.s 38%) which is added to the 1st stage results in a tremendous increase of the dynamic protein binding capacity (from 80 to 140 g, i.e. 75%). These results clearly show that with the Sartobind multistage concept described above the module area and geometry is easily adapted to the specific separation problem.

4.3. Linear salt gradient elution

This experiment clearly shows the applicability of

Table 3
Dimensions of the modules used for gradient elution experiments

Module	Module type[a]	No. of layers	Module length (cm)	Membrane area (m^2)
1	S-10K-60-6	60	6.2	1
2	S-10K-30-12	30	12.5	1
3	S-10K-15-25	15	25	1

[a] Module type characterizes membrane chemistry (S for sulfonic acid), membrane area 10 000 cm^2 followed by the number of layers and the length in centimeters.

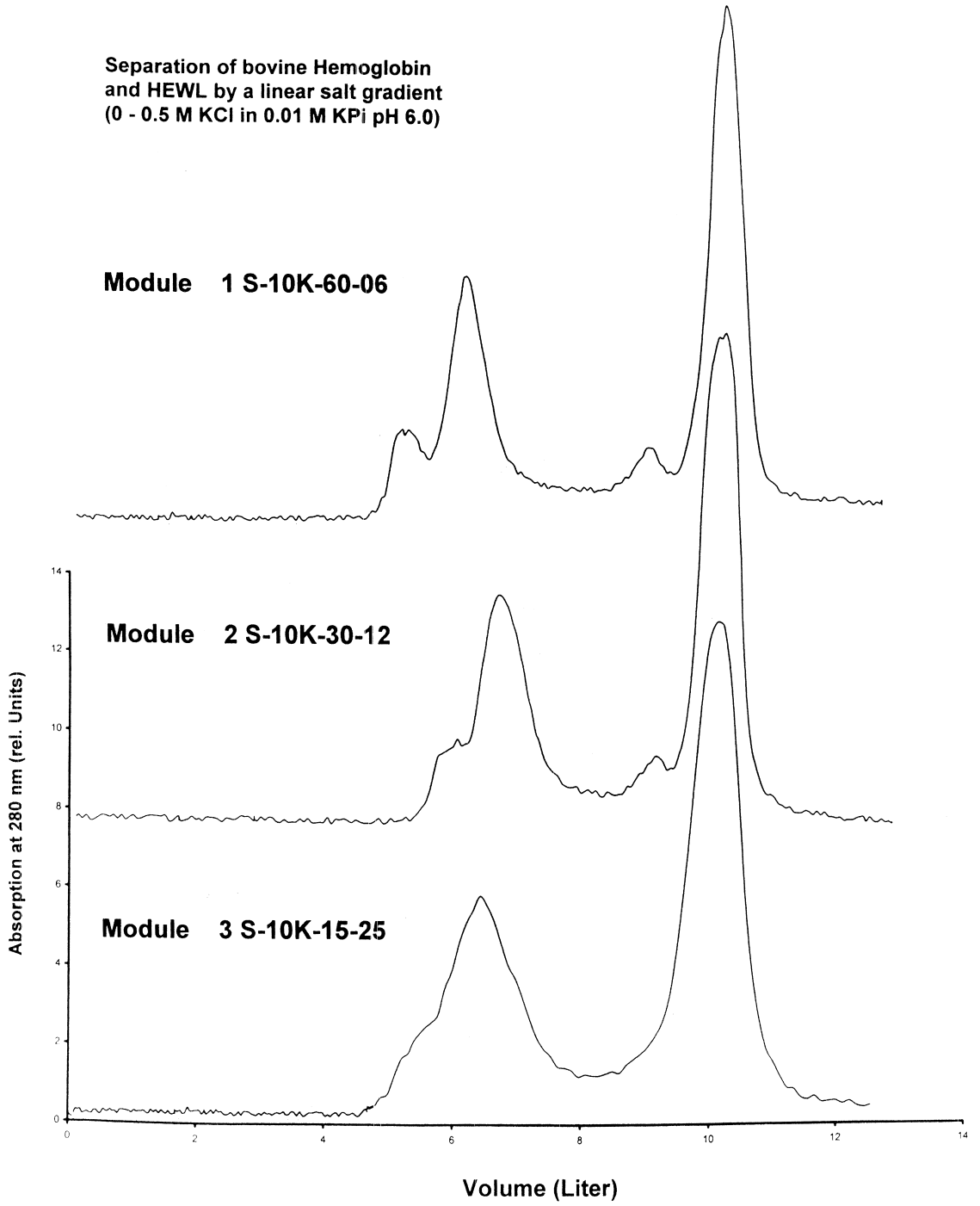

Fig. 4. Gradient elution with Sartobind modules of equal membrane areas (1 m^2) and different number of layers (from top down: 60, 30 and 15).

gradient elution to Sartobind cylindrical modules. As was expected an increase in the number of layers is accompanied by an increase in selectivity, indicated by a sharpening of the peaks and by the emergence of additional ones (Fig. 4).

5. Conclusions

A new system of adsorptive membranes units for the large scale separation of biomolecules is presented. It consists of three basic types of modules with 60, 30 and 15 membrane layers and 8, 4 and 2 m^2 membrane area, respectively. Strongly acidic and basic ion-exchange modules are available to date. Connecting modules in parallel increases capacity and flux on the sacrifice of performance. On the level of a pilot plant of 21 m^2 it has been shown that this can be overcome by combinations of parallel and serial connections. By carefully balancing these features multistage plants can be adopted to the specific demands of a given application. It has also been demonstrated that gradient elution is applicable to the module geometry chosen.

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