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Membrane Chromatography: Performance and Scale-up

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Conventional methods of chromatographic protein purification usually are based on particulate matrices. On a preparative scale, porous particles are used, where most of the ligands for protein adsorption are located on the interior surface of the adsorbents, *e.g.*, in dead-ended pores. The dominating mass transport mechanism in this case is the diffusion of the proteins in the pores, which usually is a slow process. Additionally, these matrices have to be packed in columns, where a minimum length of the packing is required to make up for packing abnormalities, resulting in packing lengths, which are significantly higher than the optimum length required for the respective separation task. For the preparative scale, this means a restriced flow rate due to pressure drop limitations. Summarising, the performance of large-scale chromatography is often characterised by long cycle times due to slow adsorption dynamics and restrictions of the column packing.

An alternative procedure is the use of microporous membranes as stationary phases for protein chromatography [1][2]. Ligands for protein adsorption are attached to the internal surface of the microfiltration membranes, which is located in the through-flown pores of the membranes. Thus, all the ligands are reached by convective flow and pore diffusion as the dominating mass transport mechanism is eliminated, thus leading to an essentially faster purification process [3]. This situation is pointed out in *Fig. 1*.

The major problem of membrane adsorbents is, that a monolayer coverage of the convective pores of a microfiltration membrane provides only insufficient ligand density per adsorbent volume due to the fact that the specific surface of these adsorbents is low compared to porous particles. To circumvent this problem, polymer-grafted chains bearing ligands are attached to the internal membrane surface, thus providing a three-dimensional ligand layer [4–6]. With this modified configuration capacities per unit volume similar to conventional adsorbents are obtained as demonstrated in *Fig. 2* for a cation-exchange membrane (Sartobind S, *Sartorius*, Göttingen, Germany).

By introducing the three-dimensional ligand sphere the above-mentioned superior mass transport capability, however, is compromised. Depending on the density and degree of cross-linking of the ligand chains, a solid-diffusion or pore-diffusion mechanism governs protein adsorption, so that overall similar transport performance is achieved as it is the case in so-called FastFlow particulate matrices. Nevertheless, linear flow rates of up to 10 cm/min may be applied without reduction in dynamic capacity of the new adsorbents [6]. An additional advantage of modified microfiltration membranes in protein chromatography is, that the material is provided in flat sheets, which are cast in one piece and may be stacked to achieve the optimum length of the packing without the need of increased column length to make up for packing irregularities. We determined the optimum packing length to be 0.6 cm, which corresponds to a stack of 30

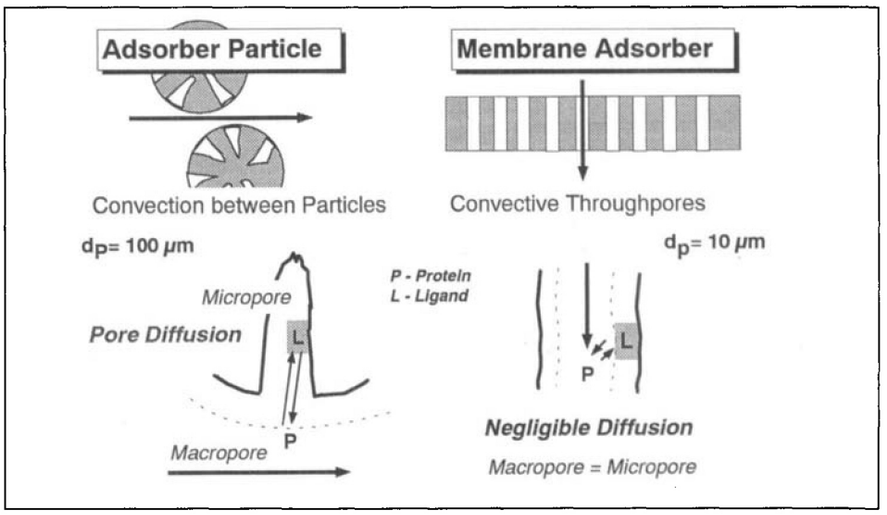

Fig. 1. *Comparing membrane- and particle-based chromatography*

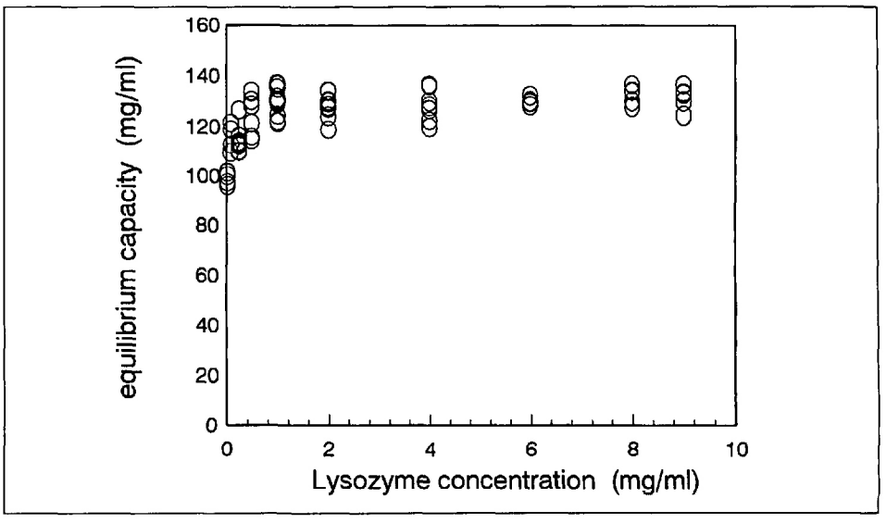

Fig. 2. *Binding isotherm of lysozyme to Sartobind S membrane absorbent* (25 mM AcONa, pH 4.5, 25°)

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membrane sheets (membrane thickness 200 µm). Provided the liquid distribution at the inlet to the stack is optimised, this very low column length allows very high liquid flow rates and therefore leads to short cycle times of the chromatographic separation.

These advantages were exploited in the purification of Human Serum Albumin (HSA) from plasma. HSA is a bulk protein product which is produced in 300–500 t per year, the major part of it being purified by the traditional *Cohn* fractionation method, but a well-described two-step chromatographic procedure is used as well [7]. Based on this process, we designed a membrane method employing a sequence of anion- and cation-exchange membranes (Sartobind Q and S, *Sartorius*, Göttingen, Germany), which is shown in *Fig. 3*. Since essentially the same raw material was used in both chromatographic modes (pooled human plasma), a valid comparison of the methods is possible. HSA isolation using modified membranes was operated successfully on the laboratory scale showing a ninefold increase in HSA productivity compared to the process based on porous adsorbent particles (220 g HSA/l of membrane adsorbent and hour compared to 26 g HSA/l of Sepharose FastFlow and hour as described by *Berglöf et al.* [8]). A scale-up to a total membrane volume of 110 ml showed a decline in productivity, the values reached, however, still were fourfold higher compared to the process mentioned above (79 g HSA/l of membrane adsorbent and hour). The reduced performance on the large scale could be attributed to the constructive details of the module which was used to stack the membrane. A large dead volume and a non-optimal liquid distribution caused the reduction in process performance. The membrane method, however, still significantly outscored the particle-based chromatography due to the short adsorbent length and the short cycle times achieved. Additionally, the method showed excellent stability and reproducibility, during HSA purification 50 successive chromatographic cycles could be conducted with no reduction in resolution or capacity [9].

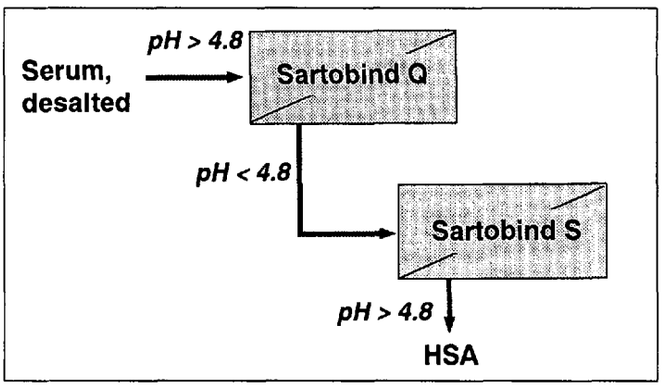

Fig. 3. *Principle of the tandem ion-exchange HSA purification employing membrane absorbents*

Summarising, membrane adsorbents may be an alternative to traditional chromatographic matrices especially for large-scale applications. By introducing a sphere of polymer-grafted ligands to the internal surface of the membrane, capacities similar to porous particles can be obtained. The continuity of the phase allows the use of thin stacks of membrane sheets at optimised column length, thus permitting high linear flow rates and short cycle times without a loss in capacity and resolution.

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Cross-Linked Enzyme Crystals as Novel Materials for Catalysis and Chromatography

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Cross-Linked Enzyme Crystals (CLEC®) [1] offer a unique combination of features normally associated with both enzymes (high activity and selectivity, ability to function under mild reaction conditions, ease of disposal) and heterogeneous catalysts (stability in different environments, recycling). This set of properties makes CLEC catalysts extremely useful in organic synthesis. Until recently, the use of crystalline enzymes was limited due to widely held misconceptions. These include 1) the perceived difficulties of large-scale protein crystal preparation, 2) the perceived mechanical fragility of protein crystals, and 3) the expected reduction in reaction rates over solution state reactions. Our recent research has demonstrated that these misconceptions are ill founded.

Indeed, five CLEC catalysts of lipases from *Candida rugosa*, *Pseudomonas cepacia*, thermolysin, subtilisin, and penicillin acylase have been manufactured on a multi-kilogram scale and commercialized by *Altus Biologics Inc.*, and many

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