Membrane Chromatography: Performance and Scale-up

Klaus H. Gebauer, Jörg Thömmes and Maria-Regina Kula*

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 restricted 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 sphere [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 adsorbent (25 mM AcONa, pH 4.5, 25°)
Cross-Linked Enzyme Crystals as Novel Materials for Catalysis and Chromatography

Alexey L. Margolin*

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

*Correspondence: Dr. A.L. Margolin
Altus Biologics Inc.
40 Allston St.
Cambridge, MA 02139, USA