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Lysozyme fractionation from egg white at pilot scale by means of tangential flow membrane adsorbers: Investigation of the flow conditions

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ABSTRACT

The application of membrane adsorbers instead of classical packed bed columns for protein fractionation is still a growing field. In the case of egg white protein fractionation, the application of classical chromatography is additionally limited due to its high viscosity that impairs filtration. By using tangential flow membrane adsorbers as stationary phase this limiting factor can be left out, as they can be loaded with particle containing substrates. The flow conditions existing in tangential flow membrane adsorbers are not fully understood yet. Thus, the aim of the present study was to gain a deeper understanding of the transport mechanisms in tangential flow membrane adsorbers. It was found that loading in recirculation mode instead of single pass mode increased the binding capacity (0.39 vs. 0.52 mg cm^{-2}). Further, it was shown that either higher flow rates (0.39 mg cm^{-2} vs. 0.57 mg cm^{-2} at 1 CV min^{-1} or 20 CV min^{-1}, respectively) or higher amounts of the target protein in the feed (0.24 mg cm^{-2} vs. 0.85 mg cm^{-2} for 2.5 or 39.0 g lysozyme, respectively) led to more protein binding. These results show that, in contrast to radial flow or flat sheet membrane adsorbers, the transport in tangential flow membrane adsorbers is not purely based on convection, but on a mix of convection and diffusion. Additionally, investigations concerning the influence of fouling formation were performed that can lead to transport limitations. It was found that this impact is neglectable. It can be concluded that the usage of tangential flow membrane adsorbers is very recommendable for egg white protein fractionations, although the transport is partly diffusion-limited.

1. Introduction

Egg white is a raw material that consists of many bio-functional proteins. The most popular one is the hydrolase lysozyme. It represents 3.5% of total egg white proteins. Due to its ability to cleave the β-1-4-glycosidic bonds of bacterial cell walls, it is often used as an antimicrobial agent, mainly against gram-positive bacteria. Resulting practical applications are in the food packaging industry, the food industry as well as in the pharmaceutical and medical area [1]. As a consequence, there is an interest in large scale fractionation of lysozyme. Classical chromatographic processes are impaired by the highly-viscous, gel-like structure of egg white. Up to now, egg white was mainly pre-treated by a precipitation step before fractionation to solve these limitations [2–4]. Although this leads to the desired egg white characteristics for subsequent chromatographic processes, it decreases the value of the remaining egg white enormously, due to significant protein losses. In our previous publications, we worked on processes to decrease the viscosity of egg white and to enable its filterability by mechanical pre-treatments [5–7]. Thereby, it is possible to work with classical chromatography systems and to retain the value of the remaining egg white simultaneously.

Several alternatives of stationary phases, away from the classical packed bed columns, have been developed during the last decades. The main aspect was to overcome the limitations resulting from the diffusion dependent transport mechanisms. The focus of the present work is on membrane adsorbers, which consist of a porous membrane with ligands linked to it. These membranes already were subject to various investigations [8–10]. Meanwhile membrane adsorbers vary in type of construction [11–14]. In the beginning, membrane adsorbers with stacked layers resulting in an axial flow were generated. This was found to result in inho-

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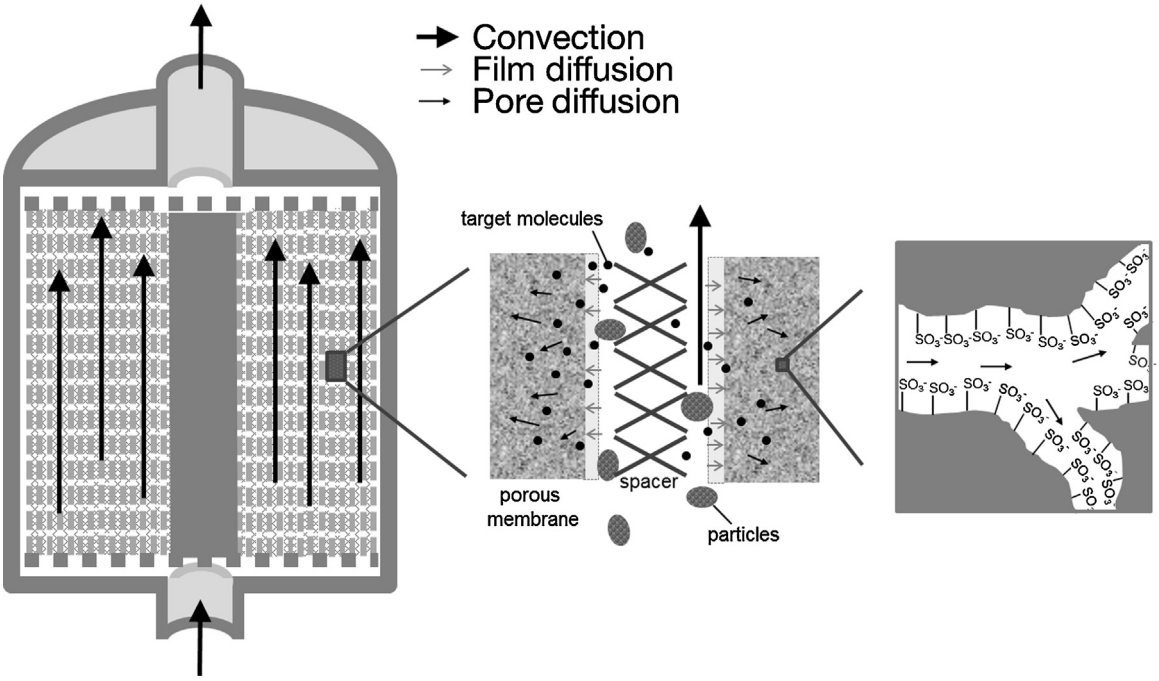

Fig. 1. Scheme of tangential flow membrane adsorbers; the first level clarifies the overall adsorber construction with the distribution plate above and below the coiled membrane, the second level illustrates the spacer net in between the porous membrane sheets and the resulting transport of the target molecules and the particles, the third level shows ligands that are bound inside the pores of the membrane; the different kinds of flow (convection, film diffusion, pore diffusion) are indicated with arrows.

mogeneous flow distribution at high flow rates, and therefore, limitations in binding [15]. Afterwards, membrane adsorbers with coiled membranes were developed, in which the flow is forced radially through the pores of the membrane layers. In our earlier work it was shown that it is very effective to work with these radial flow membrane adsorbers. Disadvantageous is the occurrence of pore blocking and high back pressure, due to the flow regime. A preceding microfiltration step is inevitable. Although the pre-treatment makes egg white filterable, microfiltration of egg white is still a limiting and time consuming factor. The third kind of membrane adsorber construction is also based on a coiled porous membrane, but with an extruded polymer spacer net between the membrane sheets to realize a constant gap of 250 μm (Fig. 1). For this kind of membrane adsorber different spacer materials and gap sizes were tested. Schoenbeck et al. [16] found that the named combination leads to the highest binding capacities. The flow is led tangentially along the membrane surface instead of through the pores. These tangential flow membrane adsorbers can be used for unfiltered, particle-containing feed solutions.

Previous works on membrane adsorbers stated a lower binding capacity compared to conventional bead based columns resulting from the lower ligand density. However, tangential flow membrane adsorbers comprise some clear advantages like an easier handling, enormous time saving by leaving out the filtration step and the possibility to apply higher flow rates. These aspects counterbalance the previous named downside especially in large scale applications [17–20]. Tangential flow membrane adsorbers open the possibility to fractionate proteins in egg white in pilot scale without protein losses caused by a protein precipitation or micro-filtration step, which would be a substantial point of difference of tangential flow membrane adsorbers. So far, no system is known that can realize that.

In general, three forms of transport mechanisms can occur during chromatographic processes, namely convection, film diffusion and pore diffusion. In stacked membrane adsorbers the flow is convective. However, different authors [15,21,22] found a flow rate dependency resulting from inhomogeneous flow distributions. Using radial flow membrane adsorbers, the transport is also based on a convective flow without diffusional limitations. Due to the uniform flow distribution, the binding capacity, purity as well as yield were independent of the flow rate, as shown in our previous work about lysozyme fractionation in lab scale [23]. Additionally, fractionation capacity using radial flow membrane adsorbers is independent of the applied protein concentration. These facts confirm that diffusion can be neglected. In the case of tangential flow membrane adsorbers, the convective flow is led tangentially along the coiled membrane. This generates convectively enhanced contacts between the molecules and the ligands on the outer surface. In contrast to radial flow membrane adsorbers the pores are not actively reached by convective flow. The protein transport to the ligands inside the pores is presumably rather based on diffusional transport mechanisms. If this is the case, an increasing flow rate should minimize film diffusion, and increase the binding capacity. A higher protein concentration should then improve pore diffusion, according to Fick's law (Eq. (1)), and therefore, increase the binding capacity.

$$J = -D\frac{\Delta c}{\Delta x} \quad (1)$$

where J [mol m^{-2} s^{-1}] is the flux, D [m^2 s^{-1}] the diffusion coefficient, $\Delta c/\Delta x$ [mol m^{-4}] the concentration gradient. However, the transport mechanisms existing in tangential flow membrane adsorbers have not been identified yet.

The flow distribution in conventional and novel chromatographic systems remains one of the major concerns. The aim of the present study was to gain a deeper understanding of the fractionation process of egg white using tangential flow membrane adsorbers. For comprehension regarding the transport mechanisms existing in tangential flow membrane adsorbers, lysozyme fractionation was carried out at different loading modes and flow rates as well as in dependence of the protein amount of the feed solution. By these results, it was possible to evaluate the impact of diffusional transport on the protein binding. Besides, the impact of possibly occurring membrane fouling on the process was assessed

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