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Impact of the substrate viscosity, potentially interfering proteins and further sample characteristics on the ion exchange efficiency of tangential flow membrane adsorbers

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ABSTRACT

The use of tangential flow membrane adsorbers is a highly recommendable option for chromatographic separations of particle containing substrates or substrates with high viscosities, like egg white. Due to the direction of the convective flow in this stationary phase, the sample is not required to be filtered. Our earlier study investigated the flow conditions of tangential flow membrane adsorbers. There, it became clear that the flow is based on a mix of convective and diffusive mass transfer. The diffusive component of the transport of the target proteins into the membrane structure depends on the viscosity of the medium according to the Stokes–Einstein equation. Therefore, it was the aim of the present study to assess the impact of viscosity on the ion exchange efficiency. Further, the amount of the main target protein of egg white, the bio-functional protein lysozyme, is relatively low compared to the other major egg white proteins. As a high amount of non-target proteins in the substrate can also interfere with the diffusional mass transfer of the target protein, it was an additional aim to understand the impact of the substrate composition on the lysozyme adsorption.

It was shown that the effective diffusion coefficients (D_{eff}) as well as the maximal binding capacities (q_{max}) strongly depend on the egg white viscosity. Applying natural egg white with a mean viscosity of 70 mPa s, a q_{max} of 0.14 mg cm^{-2} with a D_{eff} of 1.5×10^{-7} cm^2 s^{-1} were achieved. If the viscosity of egg white was reduced by means of a mechanical pre-treatment, q_{max} was increased to 0.49 mg cm^{-2} with a D_{eff} of 9.2×10^{-7} cm^2 s^{-1}. Consequently, the adsorption efficiency strongly depends on the substrate viscosity. Additionally, the impact of high amounts of non-target proteins in egg white on the adsorption of the target protein lysozyme was analyzed. It was demonstrated that the other egg white proteins strongly interfere the lysozyme binding to the membrane (q_{max} of 0.49 mg cm^{-2} compared to 0.65 mg cm^{-2}). This is probably due to steric effects and interactions between the proteins. Finally, it can be concluded that diffusion is an important transport mechanism of tangential flow membrane adsorbers, and if this is disturbed by inappropriate conditions, the process works less well.

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1. Introduction

The protein content of egg white constitutes ~90% of its dry matter. Thus, egg white is a very pure protein source. The major egg white proteins and some characteristics are shown in Table 1. It becomes clear that they vary in relative content, molecular weight as well as in their isoelectric points (IP). The egg white protein lysozyme is well known due to its broad bio-functionalities (Fleming, 1922; Wasserfall and Teuber, 1979; Cisani et al., 1984; Appendini and Hotchkiss, 1997; Gerbaux et al., 1997; Daeschel et al., 1999; Tenovuo, 2002; Conte et al., 2006; Lesnierowski and Kijowski, 2007; Lee et al., 2009). Hence, it is the aim of many works to isolate lysozyme from egg white, although with a relative content of 3.5%, its amount is relatively low compared to the other major egg white proteins (Table 1). The usage of chromatography for the isolation of proteins offers highly specific separations, especially because of the high IP of lysozyme as compared to the other egg white proteins (Table 1). However, the physical characteristics of egg white, mainly the gel-like structure with a high viscosity and the fact that it is not filterable in its natural form impair or even inhibit the usage of classical column chromatography (Levison, 2003).

Egg white possess a viscosity of around 70 mPa s (Brand et al., 2014) combined with an inhomogeneous viscosity distribution within the egg white. This is due to the fibrillar protein ovomucin that is cross-linked by intermolecular disulfide bridges, which results in a protein network structure (Donovan et al., 1970; Rabouille et al., 1990; Ternes et al., 1994). Ovomucin comprises two subunits α-ovomucin and β-ovomucin. Depending on the exact composition of these two subunits, ovomucin exists in a soluble and an insoluble form (Hayakawa and Sato, 1976, 1977). The uneven distribution of them is responsible for the inhomogeneous viscosity of egg white. Directly adjacent to the egg membrane and around the egg yolk, egg white viscosity is low. Here soluble ovomucin dominates. In between there is a high-viscous layer, comprised of a mixture of soluble and insoluble ovomucin (Sato and Hayakawa, 1977; Stadelman and Cotterill, 1995; Hiidenhovi, 2007). Thus, the egg white protein ovomucin is responsible for the impaired handling of egg white in chromatographic separations. This is the reason why nearly all of the approaches published so far are carried out with precipitated egg white as substrate. Although some of the authors, e.g. Awadé et al. (1994), Vachier et al. (1995), Guérin-Dubiard et al. (2005), Tankrathok et al. (2009), Omana et al. (2010) or Yan et al. (2011) achieve good results using this kind of pre-treatment, it leads to immense protein losses and dilution effects, whereby the value of the egg white decreases.

Up to now, a high variety of stationary phases for chromatographic processes are available. Kreuß et al. (2008) compared the performance of different conventional bead-based phases with a radial flow membrane adsorber. They found that the separation speed was faster by a factor of four using this kind membrane adsorber. However, the level of binding capacity and resolution was lower. Unfortunately, information concerning potential differences in purity are missing in this publication. Additionally Orr et al. (2013) and Ghosh (2002) summarized the differences between the various kinds of stationary phases. Overall, a disadvantage of radial flow membrane adsorbers and of conventional packed bed columns that cannot be neglected is, that for both methods, the substrate must be pre-filtered to avoid pore blocking, which impairs their usage for egg white. Previous work with membrane adsorbers with flow of the substrate through the membrane pores has shown that the reduction of viscosity of egg white is required for ion exchange chromatographic egg white protein fractionation. Thus, egg white was pre-treated prior to fractionation so far. In our studies the viscosity decrease was realized by a destruction of the ovomucin network with mechanical devices (Brand et al., 2014, 2015, 2016a; Brand and Kulozik, 2016). Tangential flow membrane adsorbers (e.g. Sartobind® Direct Capture, Sartorius Stedim, Göttingen) on the other hand theoretically allow to handle crude products containing particles or to cope with high viscosities because no flow through membrane pores takes place. A scheme of this unit can be seen in Fig. 1. The functional groups are covalently bound to a porous spirally wound membrane. SEM and CLSM images of the membrane can be seen in the publication of Wang et al. (2008). In between these membrane sheets a spacer net creates a distance of 250 μm. The product flows tangentially through this channel. The target molecules diffuse into the membrane structure where they bind to the ion exchange ligands. Since there is no convective flow through the pores of the membrane (3–5 μm), there is no risk of pore blocking, and hence, the sample is not required to be pre-filtered. However, the diffusive transport of the target proteins into the membrane structure depends on the viscosity of the medium according to the Stokes–Einstein equation (Eq. (1)) (Carta and Jungbauer, 2010). The lower the viscosity is the higher is the diffusion coefficient.

$$D = \frac{k_B T}{6\pi R_0} \cdot \frac{1}{\eta} \quad (1)$$

With D [$m^2\,s^{-1}$] as the diffusion coefficient, k_B [$J\,K^{-1}$] the Boltzmann constant, T [K] the temperature, R_0 [m] the hydrodynamic radius and η [Pa s] the viscosity.

Thus, despite the fact that the fluid flow through the annular flow path between the coiled membrane sheets is possible, the mass transfer of the target proteins still depends on the viscosity of the product. Therefore, it was the aim of this study to assess the impact of viscosity on ion exchange efficiency, i.e. the impact of a mechanical pre-treatment on the reduction of viscosity and, thus, on the diffusional mass transfer of the target proteins to the ligands located at or in the membranes. A further point of interest was to evaluate the impact of the high amounts of non-target egg white proteins (96.5% of the egg white proteins) on the diffusional mass transfer of lysozyme, and thus, on its adsorption efficiency.

2. Experimental

2.1. Substrate

2.1.1. Egg white preparation

Freshly laid eggs (hens: Lohmann Tradition) were collected from the University's research farm (Thalhausen). The egg white was separated manually from the egg yolk and the chalaza was removed. To obtain different viscosities, the egg white was pre-treated with different shear devices according to our previous publications (Brand et al., 2014, 2015; Brand and Kulozik, 2016). For the colloid mill treatment the Labor Pilot 2000/4 (IKA®, Staufen) was used with toothed geometries and a gap of 1.040 mm in between. For high-pressure homogenization the APV 1000 (SPX Flow Technology Rosista, Charlotte) was used. The operation was done in single stage mode. Table 2 gives an overview of the used samples as well

Table 1 – Major egg white proteins and their characteristics (Hiidenhovi, 2007; Datta et al., 2009; Omana et al., 2010; Huopalathi, 2007).

Protein	Relative content [%]	Molecular weight [kD]	IP [–]
Ovalbumin	54–58	45.0	4.5–4.8
Ovotransferrin	12–15	76.0–77.7	6.1–7.2
Ovomucoid	11	28.0	4.1
Lysozyme	3.5	14.3	10.7
Ovomucin	3.5	23,000 (insoluble) 5600–8300 (soluble)	4.7