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Performance of Mixed Matrix Membrane Adsorbers for Lysozyme Separation

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Abstract

An application of EVAL based mixed matrix membrane adsorbers containing a strongcation exchange resins (Lewatit SP 112 WS) for capturing of Lysozyme (LZ) has been investigated.The preparation and performance of the mixed matrix membrane adsorbers is studied with 1-octanol as additive. The membranes in this study are prepared by immersion precipitation out of a DMSO solution containing 14% EVAL and with or without 14% 1-octanol. All membranes contain 65% resin based on dry solids. The morphology of the membranes are characterized using scanning electron microscopy.The membrane structure demonstrated open and interconnected porous structure with large resin particles are distributed in membrane structure. The MMM adsorber proved high adsorption capacity for capturing and concentrating LZ in feed solution. The Mixed matrix membranes (MMM) concept was anticipated as a moderate process with an increasedcapacity and a maximum adsorption capacity of 166 mg LZ/g membrane. The MMM act in protein separations both as protein purifier and concentrator and is an attractive alternative for packed bed systems because of its high capacity, high throughput, robustness, and ease of scaling up.

Keywords: Mixed Matrix Membrane (MMM), Lysozyme (LZ), Adsorption, Membrane adsorber

1. Introduction

Rapid developments in biotechnology and the pharmaceutical potential of proteins are strongly demanding for reliable and efficient protein separation and purification processes. The isolation and purification of biomolecular targets is difficult. Many purification steps are involved, which cause product loss, require long process times and are expensive. The isolation and purification steps account for 50 to 80 % of the total production costs (Saxena, et al., 2009, Fee, et al., 2009). Besides purity the biomolecules have to maintain their native structure or biological activity (Saiful, et al., 2006, He, et al., 2008). Since many biologics, especially proteins (enzymes) are fragile molecules that cannot handle harsh conditions; an effective and reliable separation technique is required to eliminate product denaturation. Minimizing the amount of purification steps and speeding-up the purification process increases the compactness, improves the economics and reduces labor costs and the time to market.

The motivation to develop a new platform technology for protein purification and concentration lies in the shortcomings of the classical technologies. Adsorptive membrane chromatography has been introduced as integrative approach to reduce the number of steps within a purification process and to bypass the fundamental limitations of packed bed system. It is especially suited for treatment of large volumes of liquid containing low concentrations of target biomolecules. This approach is able to unite the coarse and the fine purification steps by embedding in microfiltration membrane. (Bhut, and Husson, 2009). Nowadays, adsorptive membrane chromatography can be prepared by two different approaches: by chemically coupled ligands to the internal surface of microporous filtration membranes (Hyötyläinen, et al, 2008, Engel, et al., 2008)and by entrapping functionalized particles (sorbent) into a macroporous polymericsupport (Lensmeyer, et al., 1995, Lingeman, et al., 1997, Avramescu, et al., 2003).

The membrane adsorber acts as a short wide chromatographic column with minimal operating pressure and maximal throughput (Figure 1). Membrane adsorbers can be employed as flat sheets, monolithic discs and hollow fibers. Since the adsorption capacity of a single membrane layer or fiber is limited, and to average out membrane heterogeneities, multiple membranes are stacked or put in series and housed within the filtration modules. The potential for continues progress and tremendous advances in sorbent technologies offer a broad application of the MMM in biotechnology and biomedical fields. As new

approach in art chromatographic techniques, there are still many interesting application of the MMM that remain to be investigated with respect to their performance in adsorptive membrane chromatography. This paper presents a case study for protein isolation using cation ion exchange based mixed matrix membrane adsorbers. This is established by embedding functionalized particles in a polymeric microporous structures, which are formed by a phase inversion process. Small particles with a high active surface area can be applied to capture target molecules. By applying these membranes, separations can be carried out under mild conditions without the use of organic solvents.

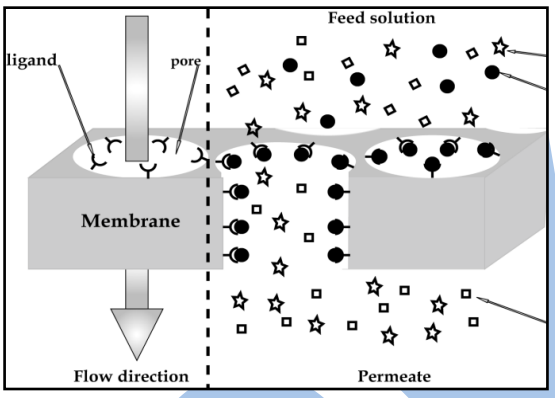

Figure 1. Illustration of membrane chromatography. (Left side of dotted line) Schematic representation of membrane chromatographyhaving ligands and porous structure. (Right side of dotted line). The Principle of membrane chromatography: biomolecules target (ligates) are selectively captured by membrane active sites (ligands) during the process load (Saiful, 2009).

2. Methods

2.1. Materials

Ethylene vinyl alcohol (EVAL) with an average ethylene content of 44 mol%, supplied by Aldrich, is used as the membrane material without further treatment. Dimethylsulfoxide (DMSO) from Merck is employed as the solvent. 1-octanol supplied by Fluka is applied as non-solvent additive in the casting solution. Tap water (H_2O) is used as nonsolvent in the coagulation bath. Ion exchange resins Lewatit SP 112 WS kindly supplied by Caldic were used as adsorbents. 10% hydrochloric acid (HCl) is used in the regeneration process. Lysozyme was used in the adsorption/desorption experiments. Lysozyme has a molecular mass of 14600 g/mol, pI around 11was supplied by Fluka. For the preparation of buffers ultrapure water obtained by a Millipore purification unit Milli-Q plus is used.

2.2 Membrane Preparation

The membranes are prepared out of a DMSO solution, containing 14% EVAL to optimise the membrane formation process, 14% 1-octanol (a non solvent additive) and 65% resin loading (Lewatit SP 112 WS). A mixture of EVAL, 1-octanol, DMSO was stirred at 50˚C until the EVAL was dissolved. Ion exchange resins were washed, dried, grinded and fractionated in order to obtain particles with a diameter smaller than 20µm. After complete dissolving of EVAL the resins were added and stirring was continued overnight to break down the clusters of particles. The solution was cast on glass plate with 0.475 mm casting knife and directly immersed in to a water bath of 40-45ºC. The formed membrane was washed for several times with tap water in order to remove the residual solvent and nonsolvent. After washing, the membranes were overnight dried in air and afterwards in a conventional oven at 50°C.

2.3 Membrane Characterization

Membrane Structure.

For the characterization of the air and glass side of the adsorber membranes, pieces of membranes are directly used whereas for the characterization of membrane cross-section pieces of membrane are wetted by water and fractured in liquid nitrogen. After the preparation samples are dried at 30ºC in vacuum oven and gold coated using a Balzers union SCD 040 sputter coater. Then the samples are examined using a Jeol TM 220A scanning electron microscope.

Membrane porosity and swelling degree.

Porosity, ε, and swelling degree, sd, are determined by calculating weight and volume of wet and dry membrane. For the membrane thickness measurements a digital micrometer of Mitutoyo is used. The results are taken from the average of four different samples. The following are the formulas to calculate porosity and swelling degree.

$$\varepsilon = \frac{M_{membrane,wet} - M_{membrane,dry}}{V_{membrane,wet}} \cdot 100 = \frac{V_{water}}{V_{membrane,wet}} \cdot 100 \quad (1)$$

$$sd(\%) = \frac{V_{membrane,wet} - V_{membrane,dry}}{V_{membrane,dry}} \cdot 100 \quad (2)$$

Where V_{dry} and V_{wet} are respectively the volume of the dry membrane and the volume of the swollen membrane after 24 hours immersing in a water bath at room temperature. Before weighing the attached water was removed by dry padding the membrane with filter paper.

Clean water flux.

Water fluxes of the membranes are determined by using nitrogen pressurised dead-end ultra filtration cell. The applied pressure is 0.1 bar. The reported flux values are measured at steady state conditions. An average flux is calculated from several experiments using different pieces of membrane.

Static experiment membrane adsorption.

Protein concentrations of 1-2 mg/ml are used in the adsorption experiments. A known weight of membrane is immersed in ultra pure water for overnight and transferred in the protein solution. The protein solutions are prepared using buffer solutions at pH 7. The ionic strength of buffer solutions used for adsorption experiments is kept constant at 17mM by adding NaCl. The samples are kept in shaking bath at 20 ºC for 24 hours. After 24 hours the absorbance of protein solution is measured using UV/vis spectrophotometer at 280nm. 2mm quartz cuvettes are used for measuring absorbance. The amount of protein adsorbed to the membrane, q_{eq} [mg protein / g membrane], at equilibrium is calculated by

$$q_{eq} = \frac{(C_0 - C_{eq}) \cdot V}{W_{membrane}} \quad (3)$$

Where C_o is the initial protein concentration [mg/ml], $C_{eq}$is the protein concentration at equilibrium [mg/ml], V is the volume of protein solution [ml] and $W_{membrane}$is the weight of the dry membrane.

Dynamic experiments.

For dynamic adsorption a stack of 10 membranes with known weight are used in the dead-end ultra filtration cell. From the static adsorption experiments we concluded that the optimal condition for protein adsorption is a solution of pH 7 and ionicstrength of 17mM. The pressure applied is 0.3 bar. In the dynamic adsorption first the membranes are equilibrated with pH 7 and I=17mM buffer. After obtaining a constant permeate flux, protein solution is applied to the dead-end filtration cell and the permeate is fractionized in samples of 2 ml. The concentration of the protein in permeate solution is determined as described earlier using the UV/Vis spectrophotometer. After reaching the break through point the experiment was stopped and the column was washed with fresh buffer to release the unbounded proteins.

3. Results and Discussion

3.1 *Membrane Characterization*

The membranes characterized are prepared out of a solution containing 14% EVAL, 14% 1-octanol in DMSO with 65% resin loading. A strong cation exchange resin (Lewatit SP112 WS), a sulphonic functionalized was applied to capture LZ in application. The amount of resin incorporated into the polymeric matrix is an important factor in the preparation of mixed matrix membrane adsorbers. In previous report (Saiful, et al.,2006, Saiful, 2009) mixed matrix membrane adsorbers prepared with 60 to 65% resin loading show optimal behavior with respect to mechanical strength and adsorption capacity. The viscosity of the casting solution with 65% Lewatit SP 112 WS resin loading is high. To cast the solution more easily on the glass plate the temperature of the casting solution is maintained at 50 ºC. Temperature of the coagulation bath is maintained at 40-45ºC. By 65% resin loading ion exchange particles occupy a very high percentage of the membrane volume, resulting in high available area for the protein adsorption. SEM pictures in the Figure 1 show the morphology of these membranes. The membranes prepared with 65% loading and 1-octanol as additive show a porous structure with interconnected pores. No finger like macrovoids across the entire cross-section. The ion exchange particles are tightly held in the polymeric matrix. No significant loss of particles is observed on the bottom surface during the membrane formation process. By adding octanol to the polymer solution the time of demixing is delayed by a factor 16, as reported by Avramescu (Avramescu, et al., 2003). Adding 14% octanol to the solution, a non-solvent, formation of macrovoids can be suppressed due to the solvation power of the

solution is reduced. The final result is that membranes prepared out of the quaternary system are mechanically more stable, sponge like structure verses bigvoids, and have higher water fluxes because of the absence of the skin layer. The membrane has porosity of 62.70 %, swelling degree of 24.60 %, and clean water flux of 401 l/h/m².

3.2. *Protein Adsorption Isotherm*

Static adsorption experiments are carried out for the adsorption of lysozyme onto the membranes. For all of the adsorption experiments the ionic strength of the protein solution is adjusted at 17mM. At higher ionic strengths the protein adsorption onto the membrane is drop because of the competition of salt ions. The adsorption isotherm for lysozyme is obtained by measuring the adsorption capacity of the membranes using different protein concentrations. A known weight of membrane is taken into 30 ml of protein solution with different concentrations. The adsorption of lysozyme is measured at pH 11, pI of lysozyme as shown in Figure 2.

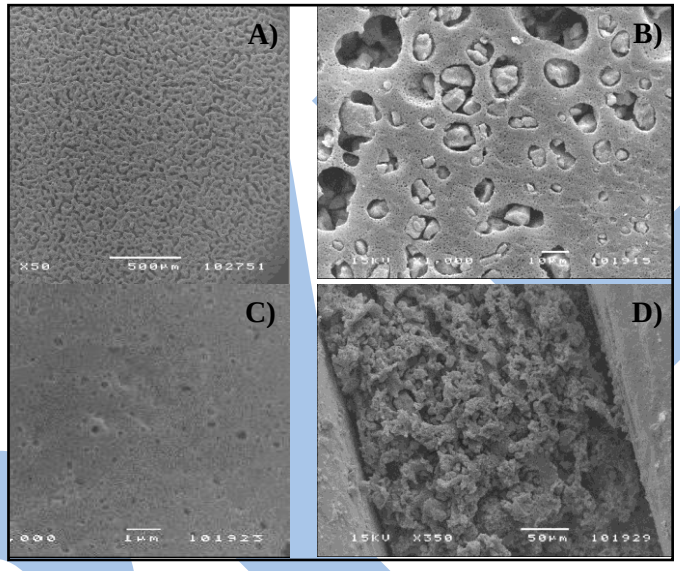

Figure 1. SEM micrograph of a EVAL based membrane adsorber containing 65% Lewatit 112 WS particles: A) Bottom-surface, magnification x50; B) Bottom-surface magnification x1500; C) Top-surface magnification x10000; D) Crossection magnifica-tion x350

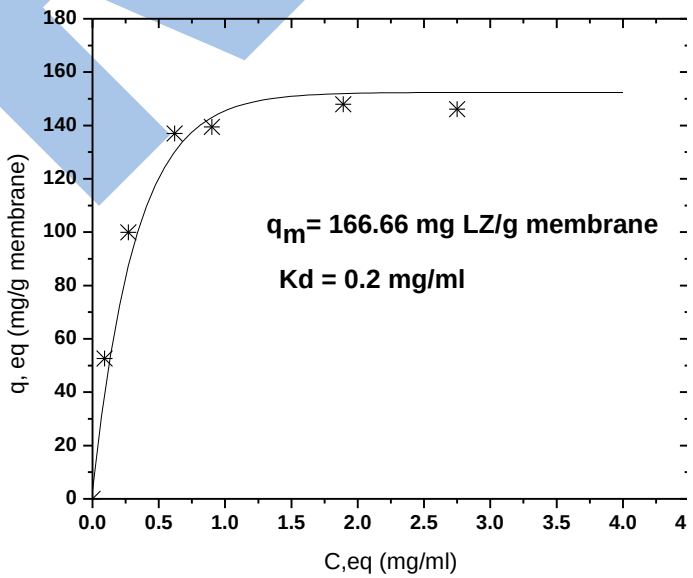

Figure 2.Correlation between the Langmuir model and experimental data of lysozyme adsorption isotherm at ionic strength 17 mM, equilibrium time 24 h. The solid line in the figure is best fitted the Langmuir isotherm

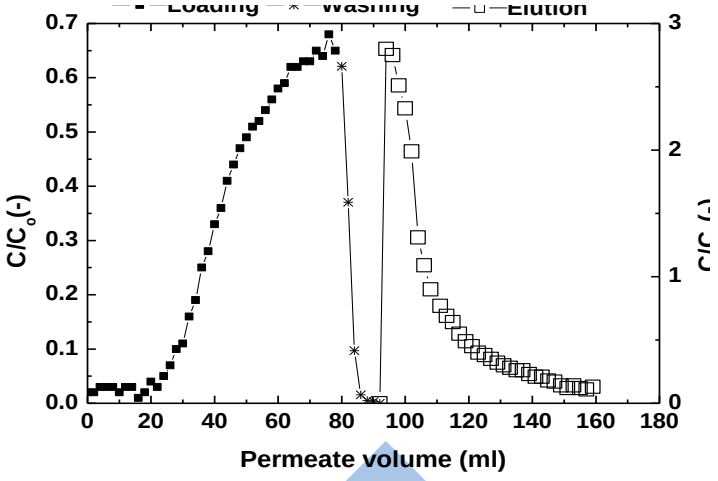

Figure 3. Typical breakthrough and elution curves of LZ for a stack of 10 MMM Lewatit 112 WS operated at a feed concentration of 2 mg/mL LZ and a constant filtration flux of 50 $Lm^{-2}h^{-1}$.

Equilibrium data were fit with the Langmuir model by nonlinear least-squares regression analysis. The solid line in the figure is the Langmuir isotherm that best fit the data. The maximum adsorption capacity (q_m) is 166.6 mg LZ/g membrane and the dissociation constant (K_d) is 0.2mg/ml. This value prove that the mixed matrix membrane adsorbersposses a good accessibility of LZ to the adsorptive site. The adsorption capacities are high compared with published data in literature. Saiful et al. (2006) obtained the adsorption capacity LZ on mixed matrix membrane incorporated with a weak cation exchange resin was 222 mg/g membrane. LZ adsorption capacity of a dye-immobilized membrane was 13.3 mg/ml (Senel, et al., 2002). The high capacity of the described MMM adsorbers can be attributed to the fact that the surface over volume is high when small sized ion exchange particles in high loadings are embedded. Lysozyme adsorption takes place mainly at the outer surface of the particles: when instead of small particles big porous particles are used the area available for adsorption is much lower (Saiful, et al., 2006).

3.3 Dynamic Adsorption of LZ on Membrane Lewatit 112 WS

In the dynamic adsorption process stack of membranes is used to increase the adsorption capacity. Avoid channeling of membranes is also a reason to use stack of membranes. The transport of the protein to the adsorptive surface in dynamic process is a function of flow conditions, protein pore diffusion as well as protein intraparticle diffusion. The pressure applied is 0.3 bar and the measured flow rate of permeate is 50 l/h/m². This permeate flow is 2.5 times lower than water flux and is because of the protein adsorption and the viscosity of solution. The dynamic lysozyme adsorption capacity at 10% break through is determined as 43 mg lysozyme/g membrane. Typical breakthrough and elution curves for lysozyme are presented in Figure 3. The measured dynamic adsorption capacity is 3 times smaller than the static adsorption capacity. This can be contributed to a non-uniform flow distribution and resistance against masstransport rate processes. Full adsorption capacity can be achieved if the flow rate through the adsorber membrane is slow enough so that each protein molecule can diffuse to the adsorptive site and can rearrange/unfold their structure to its most favorable one before the interstitial volume continues through the membrane. Full capacity can be improved also by stacking more the membrane in module.

3.4 Desorption, Regeneration and Reuse the Membrane Lewatit 112 WS

Recovery of proteins were established by phosphate buffer at 1 M (NaCl) ionic strength. In the first cycle of the membrane was obtained high recovery. However in the desorption step, the protein is not recovered completely. For the reuse, mixed matrix membrane adsorbers are regenerated with 10% HCl, washed with ultra pure water until neutral pH. Regenerated membranes are used in the next adsorption/desorption cycle. After regeneration the protein is completely desorbed from the resin.During the LZ was completely removed from the resin(Saiful, et al., 2006). In the successive cycles higher recoveries are observed as illustrated in Figure 4. Regenerating the membrane for

three times showed no loss in adsorption capacity.

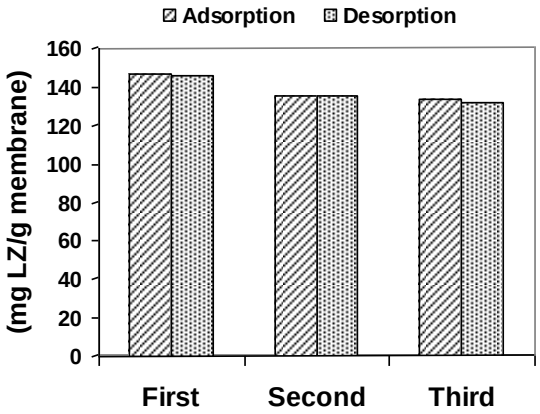

Figure 4. Static adsorption-desorption recycle membrane adsorberLewatit 112 WS at a feed concentration of 2 mg/mL LZ

4. Conclusion

The performance of MMM adsorbers for enzyme capturing and concentration was studied. The MMM adsorbers were prepared by a phase inversion method. The capacity was established by embedding of small particles lewatit 112 WS into a microporousEVAL membrane. A membrane with higher resin loading is preferable for providing a higher adsorption capacity by 65% resin based on dry solids. The membrane structure demonstrated open and interconnected porous structure with large resin particles are distributed in membrane structure.The membrane porosity was 62.70 %, 24.60 %, of swelling degree and clean water flux was 401 l/h/m². The MMM adsorbers demonstrate a high static and dynamic protein adsorption capacity and an easy scale up for the capturing of LZ.

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