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# Enzyme capturing and concentration with mixed matrix membrane adsorbers

Saiful, Z. Borneman, M. Wessling *

*Membrane Technology Group, Faculty of Science and Technology, University of Twente, P.O. Box 217, NL-7500 AE Enschede, The Netherlands*

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**Abstract**

This study reports the use of membrane adsorbers for lysozyme (LZ) capturing and concentration: the membrane adsorbers are prepared by incorporation of ion exchange resins into an EVAL porous matrix. The mixed matrix membrane (MMM) adsorber possesses an open and interconnected porous structure with a large ion exchange surface available for enzyme adsorption. The adsorptive membrane features both a high static as well as a high dynamic LZ adsorption capacity. The measured LZ adsorption isotherm is of the Langmuir type, with a maximum adsorption capacity of 147 mg LZ/ml membrane. Dynamic LZ adsorption capacity at a flux of 25 l/h/m$^2$ was 63 mg LZ/ml membrane, which is significantly higher than the equivalent commercial membrane Sartobind C. Since the kinetics of desorption processes are faster than the kinetics of adsorption processes, the performance can be improved by exerting the desorption processes at higher fluxes than the adsorption processes. The MMM can be reused in multiple adsorption/desorption cycles maintaining the high binding capacity performance. Fluorescence spectra of the LZ after adsorption and elution were similar to native LZ. This is confirmed by activity tests showing that the activity of LZ was maintained after an adsorption and desorption cycle.


*Keywords:* Mixed matrix membrane (MMM); Sartobind C; Adsorption; Lysozyme (LZ); Activity; Membrane adsorber; Ion exchange

## 1. Introduction

Chromatography is the method most widely used to obtain the required purity for biotherapeutics and diagnostics [1]. Among all the chromatographic methods, ion exchange is one of the most often used techniques in downstream processing. It is employed for recovery and purification of proteins, polypeptides, nucleic acids, polynucleotides and other biomolecules. Ion exchange is advantageous in terms of broad applicability, high resolution and large adsorption capacity in large-scale protein purification processes [2]. Another advantage of this technique is that the elution takes place under mild conditions so that the proteins maintain their native conformation during the chromatographic processing. Protein separation in ion-exchange chromatography is mainly determined by the electrostatic interaction between the solute and the oppositely charged of the surface stationary phase [3]. Many types ion exchange resins are nowadays commercial available and have been applied in protein capturing, purifying and polishing steps [4,5].

The developments in bio downstream processes are promoted by reliable and efficient separation and purification methods, for the many diverse biomolecules that are produced and studied in biopharmaceutical and bioproduct industries. Large-scale separation and purification processes must be cost efficient because they contribute for a large extend to the total product manufacturing costs. In a typical protein production process from fermentation to final product, isolation and purification account for 50–90% of the total production costs [6,7]. The desirable properties in protein purification are among others a high throughput, a high capacity and the ability of the adsorbent to be used repeatedly [8].

In the recent years, there is a considerable and increasing interest in developing membrane adsorbers. The membrane adsorber acts as a short and wide chromatographic col-

*Abbreviations:* ATCC, American type culture collection; BSA, Bovine serum albumin; CER, cation exchange resin; DMSO, dimethylsulfoxide; EVAL, ethylene-vinyl copolymer; HCl, hydrochloric acid; LZ, lysozyme; Lew, Lewatit; MMM, mixed matrix membrane; p*I*, isoelectric point; U.A., unit activity; Trp, tryptophan

* Corresponding author. Tel.: +31 53 4892951; fax: +31 53 4894611.
*E-mail address:* m.wessling@utwente.nl (M. Wessling).

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umn. Membrane adsorbers can exist in a variety of configurations [9–12] with a variety of adsorptive mechanisms (e.g. ion exchange, hydrophobic, reversed-phase or affinity interactions). The advantage of adsorptive membranes is the absence of a long diffusion path length, which often occur in fixed bed chromatography. In adsorptive membranes, the transport of the dissolved molecules to the active sites in the membrane occurs by convective flow rather than by diffusion through a stagnant fluid inside the pores of an adsorbent particle. Another major advantage of the membrane adsorbers is the relative ease of scaling up when compared with packed bed systems [13]. Membranes with adsorptive chromatography properties do exhibit technological progress in both membrane filtration and fixed-bed liquid chromatography. Membrane adsorbers combine the selectivity of chromatography resins with the high productivity of filtration membranes [10,11,13,14].

Avramescu et al. [13] described a new chromatographic concept for a single-step process for the preparation of ion exchange mixed matrix membrane (MMM) adsorbers having particulate material entrapped in a porous matrix. A mixture of dissolved polymers and additives in which particulate material was dispersed, was cast/spun and solidified by a phase inversion process into a flat/fibrous membrane. The prepared MMM contain ion exchange particles tightly held together within a porous polymeric matrix, the latter not interfering with the activity of the particles. Such membranes can be prepared in different shapes and can be operated either as stacked microporous flat sheet membranes or as modules containing fibrous membranes. By independent selection of particles and matrix material various functions such as ion exchange, adsorption, catalysis or enzymatic activity can be incorporated. Such membrane adsorbers may also serve as a platform to which an end-user can couple a specific ligate [10].

MMM adsorbers have been applied for separation and recovery of BSA, Hb and bilirubin [13,15,16]. Although several applications have been introduced to membrane adsorbers already, there are still many interesting applications that remain to be explored with respect to their performance in membrane chromatography. One of the greatest challenges in downstream purification processing is the capturing and concentration of enzymes. Many biomolecules are difficult to purify in a native and biological active form. An effective and reliable separation technique is needed to achieve this requirement. Due to their low pressure drop and mild process conditions, a MMM adsorbers are good candidates to maintain the native conformation and the biological activity of proteins or enzymes during the isolation and purification processes. In this study, we provide an extensive characterization of MMM adsorbers in static and dynamic mode for enzyme recovery. A weak cation exchange resin, Lewatit CNP80 WS, is incorporated as particulate material into a porous EVAL matrix to prepare adsorber membranes with a high particle loading. The performance of the prepared ion exchange MMM as enzyme adsorbers was investigated using lysozyme (LZ) as a model enzyme. System parameters such as membrane porosity, swelling and permeability and process conditions like pH, ionic strength and membrane flux were varied to study their effect on the performances of mixed membrane adsorbers during lysozyme recovery.

## 2. Experimental

### 2.1. Materials

EVAL (a random copolymer of ethylene and vinyl alcohol) with an average ethylene content of 44 mol% was purchased from Aldrich and was used as membrane material without further modification. Dimethylsulfoxide (DMSO, Merck) was employed as solvent and 1-octanol (Fluka) as non-solvent-additive in the casting solution. Water was used as non-solvent in the coagulation bath. Lewatit ion exchange resins CNP80 WS (Bayer) were used as adsorbent particle. The particles were grinded and fractionated down to a fraction with an average size of 10 μm. This size was chosen because of the high surface over volume ratio while the pores in the membrane are still sufficient to maintain a high flux and to avoid particle loss out of the matrix. Hydrochloric acid (HCl) is used for the regeneration of MMM adsorbers. The enzyme used for the adsorption measurements was lysozyme (LZ). LZ was obtained by Fluka and has a Mw of 14,600 g/mol and a p*I* around 11. Freeze-dried *Micrococcus lysodeikticus* cells (ATCC strain #4698) were used as enzyme substrate, purchased from Fluka biochemical. Sartobind C membranes, kindly supplied by Sartorius, were used as a representative of commercial membrane adsorbers.

Buffer solutions were freshly prepared in ultra pure water. Ultra pure water was prepared using a Millipore purification unit Milli-Q plus. The buffers used for adsorption were an acetate buffer for the pH 4–5 regime, a phosphate buffer for pH 3, 6–8 and 12 regime, a Tris buffer for pH value 9 and a carbonate buffer for pH values between 10 and 11. The ionic strength of the adsorption buffers was kept constant at 17 mM by addition of NaCl. This low ionic strength results in maximum adsorption [17]. The elution buffer was prepared by increasing the ionic strength of the adsorption buffer pH 7.0 to 0.5 M using NaCl.

### 2.2. Membrane preparation and characterization

#### 2.2.1. Membrane preparation

To obtain membranes with protein adsorptive properties, Lewatit ion exchange particles with an average diameter of 10 μm were added to a solution containing 10 wt% EVAL and 10% 1-octanol in DMSO. The 1-octanol was added to the casting solution in order to improve the membrane morphology. The mixtures were stirred over night at 50 °C to break down clusters of particles. The MMM were prepared by immersion precipitation. For this the polymeric mixture was cast (casting knife 0.475 mm) on a glass plate and immediately immersed into a water coagulation bath at 40–45 °C and the membranes were formed a few moments after immersion. The membranes were washed with tap water at room temperature to remove residual solvent and 1-octanol. After washing, the membranes were dried in the air overnight and afterwards dried in a conventional oven at 50 °C.