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Separation of protein mixtures by an integrated electro-ultrafiltration–electrodialysis process

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ABSTRACT

An integrated process for improving selectivity and permeate flux in ultrafiltration based protein fractionation was developed by combining electrodialysis (ED) with electro-ultrafiltration (EUF). The performance of such a process was first investigated using individual proteins (bovine serum albumin and lysozyme) and then with a mixture of the two. The experimental results showed that as with EUF on its own, the build-up of lysozyme concentration polarization near the cation exchange membrane in the permeate compartment significantly affected ion migration and led to change in pH and conductivity of the feed solution during an EUF–ED process with lysozyme, or bovine serum albumin and lysozyme mixture. As compared with EUF, EUF–ED of protein mixture resulted in a 20% increase in permeate flux with the lysozyme transmission remaining the same. The demineralization that occurred during EUF–ED made this process suitable for protein separation from the feed solutions with high conductivity.

1. Introduction

Downstream processing or bioseparation is an extremely important component in the manufacturing process for proteins used as food and pharmaceuticals. A protein bioseparation process should be easy to scale up, give high selectivity, and must be inexpensive. Chromatography, which is commonly used for protein purification, gives high selectivity but is expensive to operate and hard to scale-up. Membrane based techniques are scalable, give high productivity, can be optimized to give high selectivity of separation and can be quite cost-effective [1–4].

Ultrafiltration, which relies on the sieving properties of porous membranes, is widely used for concentration and fractionation of biomacromolecules such as proteins [1,4]. Fouling and concentration polarization are major problems in ultrafiltration based separation processes. The concentration polarization layer adjacent to a retaining membrane consists mainly of preferentially rejected species such as high molecular weight proteins. In some cases low molecular weight proteins may also be co-retained by larger ones, particularly when these carry opposite charges [5–7]. Whatever the mechanism of solute retention, concentration polarization results in permeate flux decline and the reduction in transmission of the protein desired in the permeate stream in a given fractionation process.

Till date, a number of techniques have been developed to alleviate the detrimental effects of concentration polarization and membrane fouling. Electric field enhanced ultrafiltration or electro-ultrafiltration (EUF) is an effective method for reducing concentration polarization and its effectiveness has been demonstrated in protein concentration [8,9], protein fractionation [5], juice clarification [10], and arsenic removal [11,12] processes. In EUF processes where the objective is the fractionation of protein mixture, the electric field moves the larger proteins away from membrane surface and thus makes it easier to transmit smaller proteins through the membrane [5]. However, for this to be effective, the isoelectric points of the proteins being fractionated should be quite different, in order to maintain significant differences in electric mobility of these proteins. Also, EUF cannot be used very effectively if the proteins to be separated have like charge at the operating condition. The choice of solution environment in an EUF process is very important since the separation relies on electrostatic charge which manifests itself best in a low ionic strength solution. Not surprisingly, for the feed solutions having high ionic strength, the efficiency of EUF is significantly reduced, since the electric field strength decreases when the current intensity remains unchanged in this case [8]. The feed solutions with high ionic strength have to be desalted before treatment by EUF. It is inconvenient and low efficient. Electrodialysis (ED), which is a

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Nomenclature

A	electrode area (m^2)
AEM	anion exchange membrane
CEM	cation exchange membrane
E_{feed}	electric field strength in the feed compartment (V/m)
ED	electrodialysis
EUF	electro-ultrafiltration
EUF–ED	electro-ultrafiltration–electrodialysis
h	height of the feed compartment (m)
I	current (A)
J_t	permeate flux of the feed solution at the end of each run (10^{-6} m/s)
J_0	buffer permeate flux before the experiment (10^{-6} m/s)
J_1	buffer permeate flux after the experiment (10^{-6} m/s)
Q_t	permeate volumetric flow rate of the feed solution at the end of each run (m^3/s)
Q_0	permeate volumetric flow rates of buffer before the experiment (m^3/s)
Q_1	permeate volumetric flow rates of buffer after the experiment (m^3/s)
R_c	concentration polarization resistance ($\times 10^{-12}$/m)
R_f	fouling resistance ($\times 10^{-12}$/m)
R_{fE}	electrodialysis resistance ($\times 10^{-12}$/m)
R_{feed}	resistance of the feed compartment (Ω)
R_m	membrane resistance ($\times 10^{-12}$/m)
R_t	total resistance ($\times 10^{-12}$/m)
u	cross-flow velocity (m/s)
U_{feed}	electric potential at the feed side (V)
UFM	ultrafiltration membrane

Greek symbols

κ_{feed}	conductivity of the feed solution (S/m)

technique that uses cation and anion exchange membranes placed within an electric field is widely used for salt removal [13–18]. More recently, the use of ultrafiltration in conjunction with electrodialysis has been examined for the separation of bioactive peptides [19,20]. If ED is introduced to the EUF process, separation of molecules and removal of salts, which are both driven by the electric field, could be achieved at the same time. Thus, the feed solutions with high ionic strength could be treated by this integrated method directly and the desalination before the conventional EUF is removed. The integrated method could also enhance the separation of molecules in the feed solutions with low ionic strength by providing higher electric field strength, since the ionic strength is further reduced in this process. Therefore, to integrate ED with EUF could achieve better performance than conventional EUF for the feed solutions with high or low ionic strength, extending the application of membrane process driven by electric field.

Integration of multiple separation technique into one could potentially improve both separation efficiency and product throughput [21–23]. In the present study, the coupling of ED with EUF for efficient protein fractionation was examined. Bovine serum albumin (BSA) and lysozyme were used as model proteins and the fractionation of binary mixtures of the two was performed by the integrated electro-ultrafiltration–electrodialysis (EUF–ED) process based on the use of a 30 KDa ultrafiltration membrane.

The objectives of the present work were:

1. To evaluate the feasibility of using EUF–ED for protein fractionation.
2. Assessment of filtration performances using single protein (either BSA or lysozyme) and binary mixture of the two.
3. Study of the effects of pH and conductivity variations induced by ion migration on permeate flux during the EUF–ED process.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, >98%, catalog # A-7030) was purchased from Sigma Chemical, St Louis, MO, USA. Lysozyme (ultra pure grade, catalog # 0663) was purchased from Amresco Inc., Solon, OH, USA. Properties of the two proteins used in this study above are shown in Table 1. Carbonate buffer (20 mM ionic strength, pH 10) was prepared by mixing 10 mM solutions of Na_2CO_3 with $NaHCO_3$ (both purchased from Beijing Chemical Works, China) to obtain the appropriate pH followed by the addition of NaCl (purchased from Guangdong Xilong Chemical Co., Ltd.) such that its effective concentration in the buffer was 10 mM. Phosphate buffer (50 mM ionic strength, pH 7.4) was prepared by dissolving required quantities of Na_2HPO_4 with NaH_2PO_4 to obtain the appropriate pH and ionic strength. Protein solutions were prepared by slowly adding pre-weighed quantities of protein powder into buffer solution.

2.2. EUF–ED rig

The EUF–ED module was made of polycarbonate and was fabricated in our laboratory. The arrangement of membranes and electrodes within the module is shown in Fig. 1b. Both, a DF-120 cation exchange membrane and a DF-120 anion exchange membrane (sourced from Shandong Tianwei Membrane Technology Company, China) were used in our set-up. The properties of the ion exchange membranes were listed in Table 2. Therefore it was significantly different from a conventional EUF unit (see Fig. 1a for comparison) which would use two cation or two anion exchange membranes. Also, unlike in a conventional electrodialysis module, the content of the permeate compartment was not re-circulated and the trans-membrane pressure was generated on the feed side. The module consisted of four compartments, separated by a cation exchange membrane on the side of cathode, a 30 KDa molecular weight cut-off polyethersolfone membrane (Shanghai Institute of Applied Physics, China Academy of Sciences, China) in the middle, and an anion exchange membrane on the side of anode. The heights of the anodic, feed, permeate and cathodic compartment were 9, 3, 3, and 3 mm. The effective membrane area was 35 cm^2. The membranes were supported by polycarbonate plates which were drilled with uniformly distributed cylindrical pores. Both the anode and cathode consists of ruthenium coated titanium electrodes (produced by Beijing Hengli Titanium Industry Co., Ltd., China).

The EUF–ED rig is shown in Fig. 2. The electrolyte (50 mM sodium phosphate buffer, pH 7.4) and the feed solution were

Table 1
Properties of proteins used in this study [5].

Property	BSA	Lysozyme
Molecular weight (kg/mol)	69	14.6
Isoelectric point	4.7	11.0