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Fractionation of proteins with two-sided electro-ultrafiltration

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Abstract

Downstream processing is a major challenge in bioprocess industry due to the high complexity of bio-suspensions itself, the low concentration of the product and the stress sensitivity of the valuable target molecules. A multitude of unit operations have to be joined together to achieve an acceptable purity and concentration of the product. Since each of the unit operations leads to a certain product loss, one important aim in downstream-research is the combination of different separation principles into one unit operation. In the current work a dead-end membrane process is combined with an electrophoresis operation. In the past this concept has proven successfully for the concentration of biopolymers. The present work shows that using different ultrafiltration membranes in a two-sided electro-filter apparatus with flushed electrodes brought significant enhancement of the protein fractionation process. Due to electrophoretic effects, the filtration velocity could be kept on a very high level for a long time, furthermore, the selectivity of a binary separation process carried out exemplarily for bovine serum albumin (BSA) and lysozyme (LZ) could be greatly increased; in the current case up to a value of more than 800.

Thus the new two-sided electro-ultrafiltration technique achieves both high product purity and short separation times.

Keywords: Electrofiltration; Ultrafiltration; Fractionation; Dead-end-filtration; Proteins

1. Introduction

Downstream processing is one of the most cost-intensive parts in the bioproduction of proteins. Applicants require a certain protein or protein group out of a complex mixture without impurities and preferably complete. Therefore selectivity is one of the main factors to increase, in order to improve the separation process. Chromatography steps are highly selective but (too) expensive (Bargeman et al., 2002). Membrane fractionation is a promising tool for biotechnological

Abbreviations: BSA, bovine serum albumin; LZ, chicken egg lysozyme; PAN, polyacrylonitrile; PES, polyethersulfone; PWF, pure water flux; SEC, size exclusion chromatography; HPLC, high performance liquid chromatography; MWCO, molecular weight cut off

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Nomenclature

A	area (m^2)
B_{22}	second virial coefficient (mol ml/g^2)
c	concentration ($g\,l^{-1}$)
d	distance (m)
D	diffusion coefficient ($m^2\,s^{-1}$)
E	electric field ($V\,m^{-2}$)
F	Faraday's constant ($C\,mol^{-1}$)
I	electric current (A)
J	filtrate flux ($l\,m^{-2}\,h^{-1}$)
J_i	transport rate ($g\,m^{-2}\,h^{-1}$)
$\bar{J}$	averaged filtrate flux ($m^3\,m^{-2}\,h^{-1}$)
k	convective coupling coefficient
k_B	Boltzmann's constant ($J\,K^{-1}$)
K	force (N)
M	molecular weight ($g\,mol^{-1}$)
N_A	Avogadro's constant (mol^{-1})
p	pressure applied (bar)
r	radius (m)
R	filtration resistance (m^{-1})
t	time (s)
T	temparature (K)
u	electrophoretic mobility ($m\,mol\,N^{-1}\,s^{-1}$)
U	voltage (V)
v	velocity ($m\,s^{-1}$)
V	volume (ml)
W_{22}	pair potential of mean force (J)
x	distance (m)
z	charge ($mol\_charge\,mol^{-1}$)

Greek symbols

ε_0	permittivity of free space ($A\,s\,V^{-1}\,m^{-1}$)
ε_M	porosity of membrane
ε_r	relative permittivity
ζ	zeta potential (V)
η	dynamic viscosity ($kg\,m^{-1}\,s^{-1}$)
κ	specific conductivity ($S\,m^{-1}$)
τ_i	transmission
Φ	fractionation index ($l\,m^{-2}\,h^{-1}$)
Ψ	selectivity

Indices

disp	dispersion
eff	effective
elec	electrical
h	hydrodynamic
hs	hard-sphere
i	substance (protein) index
r	index of substance to be kept in retentate

downstream processing, but is seen as not being sufficiently selective when separating similar sized proteins. Much research work has been undertaken in membrane filtration processes, all intending to improve the operation (Bowen and Williams, 1996; Mukai et al., 1998; Ho et al., 1999; Tessier et al., 2004). Electrostatic effects that appear in the ultrafiltration process have been the center of extensive attention. Interactions are crucial for the whole process both between the different substances in the feed stream as well as between these and the membrane (Koehler et al., 2000; Maruyama et al., 2001). For instance, opposite charges of the different proteins can lead to agglomeration (Filipe and Ghosh, 2005). Therefore extensive research effort has been made to change, diminish, or use these interactions in protein fractionation processes (Higuchi et al., 1991; Saksena and Zydney, 1994; Iritani et al., 1995; Vaneijndhoven et al., 1995; Ghosh and Cui, 1998; Cheang and Zydney, 2003). Other approaches of improving protein fractionation processes targeted at changes in the process structure. Beyond small changes such as optimising transmembrane pressure or cross-flow velocity, the fractionation process was improved by employing membranes in series (Feins and Sirkar, 2004) or establishing a cascade ultrafiltration (Ghosh, 2003) which brought substantial progress. It was also found that combining several unit operations into one stage can result in high purity and recovery of the target protein (Ghosh, 2004).

Filtration kinetics are the second crucial criterion when regarding filtration processes. The use of additional forces in a hybrid process has been proclaimed by several research groups for biological (Mukai et al., 1998; Iritani et al., 2000; Bellara et al., 1997) and non-biological products (Watson and Li, 1999; Peuker and Stahl, 2002). Hofmann and Posten (2003) developed an electrofiltration concept especially for the application to biological systems such as biopolymers. They used a two-sided filtration device with