

**COMPARATIVE STUDY OF PROTEIN FRACTIONATION IN STIRRED CELL AND
CROSS FLOW ULTRAFILTRATION MODULE**

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DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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CROSS FLOW ULTRAFILTRATION MODULE**

by

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DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

Submitted

in fulfilment of the requirement of the degree of Doctor of Philosophy

To



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Certificate

This is to certify that the thesis entitled “**Comparative study of protein fractionation in stirred cell and cross flow ultrafiltration module**” being submitted by Mr. **Premnath S.** is worthy of consideration for the award of the degree of **Doctor of Philosophy**. The thesis has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi and is a record of the original bonafide research work. The results presented in this thesis have not been submitted in part or full to any other universities or institutes for the award of any other degree or diploma.

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Abstract

Ultrafiltration is the important unit operation in protein purification in biopharmaceutical industries. Most of the ultrafiltration experiments in lab scale are performed in stirred cell (SC) module because of its simpler design and uniform hydrodynamics. However, large scale works are performed in cross flow (CF) system. Hence, comparison of performance between these modules will help in scale up and scale down studies of ultrafiltration. In this study, fractionation of binary protein mixtures were performed in SC and CF systems. A basic condition of constant shear stress near membrane surface was maintained in SC and CF system for first time to carry out comparison study of protein fractionation.

Mathematical correlation for shear stress near membrane surface was developed for SC and CF ultrafiltration system. Radial inward flow (secondary liquid flow) near membrane surface was taken into account, apart from circular liquid flow (primary flow) for evaluating membrane shear stress in SC module. In case of CF flat sheet system, membrane shear was calculated by assuming liquid dynamics in CF as flow through parallel plates.

Two sets of protein mixture were chosen to compare fractionation in SC and CF systems. Ovalbumin (45 kDa, pI-4.5) and lysozyme (14.5 kDa, pI-11.4) mixture was used to study size based fractionation in 30 kDa polyethersulfone membrane (PES30). In second set of binary mixture, haemoglobin (65 kDa, pI-6.8) and ovalbumin of similar sized proteins was separated based on charge difference using 100 kDa polyethersulfone membrane (PES100). Purity, selectivity, transmission, flux and fouling were studied for broad range of hydrodynamic and physico-chemical parameters in both UF modules. Protein adsorption on membrane surface was also studied using atomic force microscopy (AFM).

Protein fractionation was studied at different hydrodynamic parameters of TMP (30, 60, 90 & 120 kPa) and membrane shear stress (0.215, 0.61, 1.715 & 3.15 Pa) in SC and CF

system. In size based fractionation, best separation of lysozyme (purity > 98%) was found at TMP of 30 kPa and membrane shear of 1.715 Pa for both UF system. Purity was less sensitive to TMP and shear condition studied. Lysozyme transmission was greater than 90 % with recovery of ~70%. At all operating condition, protein separation in SC was successfully reproduced in CF system under constant membrane shear condition. In case of charge based separation, membrane fouling had significant impact on purity and transmission of protein. Almost similar purity of haemoglobin (~60 %) was obtained at TMP of 30 kPa and 0.215 Pa shear condition in both systems. The best separation was found at low TMP and shear condition for both modes of protein fractionation.

The physico-chemical condition such as pH and ionic strength had little effect on purity for size based protein separation. Lysozyme transmission varied distinctly with pH and ionic strength of feed in both UF systems. Highest purity of lysozyme >95% was obtained with recovery of 65%. On other hand, pH and ionic strength had significant effect on protein selectivity in charge based protein separation. Selectivity of protein could be reversed by changing pH of feed solution. SC had better selectivity than CF due to secondary sieving of protein adsorbed membrane. High purity (Hb - 88% & Ova - 83%) and high recovery (~ 70%) was obtained at low ionic strength in SC module.

Flux decline with time during protein fractionation was studied for different hydrodynamic and physico-chemical condition in SC and CF module. High shear and low TMP is the best condition to keep minimum flux decline in both UF modules. Flux decline was higher in charge based fractionation compared to size based fractionation. SC pronounced more flux decline than CF under constant shear condition due to inherent difference in flow dynamics. Ionic strength and pH of feed solution had significant effect on flux decline. High flux decline was found at higher ionic strength and isoelectric point of protein due to absence of electrostatic interaction. Location based protein adsorption on SC membrane was studied

using AFM surface analysis. The high average surface roughness (R_a) at central region of circular membrane confirms high protein adsorption on membrane due to secondary radial inward flow.

सार

बायोफर्मासिटिकल उद्योगों में प्रोटीन शुद्धि में अल्ट्राफिल्ट्रेशन महत्वपूर्ण इकाई संचालन है। लैब स्केल के अधिकांश अल्ट्राफिल्ट्रेशन प्रयोगों को सरल डिजाइन और वर्दी हाइड्रोडायनामिक्स की वजह से संभ्रमित सेल (एससी) मॉड्यूल में किया जाता है। हालांकि, बड़े पैमाने पर क्रॉस प्रवाह (सीएफ) सिस्टम में प्रदर्शन किया जाता है इसलिए, इन मॉड्यूल के बीच प्रदर्शन की तुलना पैमाने पर बढ़ने और अल्ट्राफिल्ट्रेशन के अध्ययन को कम करने में मदद करेगी। इस अध्ययन में, द्विआधारी प्रोटीन मिश्रण का विभाजन एससी और सीएफ सिस्टम में किया गया था। झिल्ली सतह के पास लगातार कतरनी तनाव की एक बुनियादी स्थिति अनुसूचित जाति और सीएफ प्रणाली में पहली बार प्रोटीन विभाजन की तुलना अध्ययन करने के लिए बनाए रखा गया था।

झिल्ली सतह के पास कतरनी तनाव के लिए गणितीय संबंध एससी और सीएफ अल्ट्राफिल्ट्रेशन सिस्टम के लिए विकसित किए गए थे। एससी मॉड्यूल में झिल्ली कतरनी तनाव के मूल्यांकन के लिए परिपत्र तरल प्रवाह (प्राथमिक प्रवाह) के अलावा झिल्ली सतह के पास रेडियल आवक प्रवाह (माध्यमिक तरल प्रवाह) को ध्यान में रखा गया। सीएफ प्लैट शीट सिस्टम के मामले में, झिल्ली कतरनी सीएफ में तरल गतिशीलता को समांतर प्लेटों के माध्यम से प्रवाह के रूप में मानते हुए गणना की गई थी।

प्रोटीन मिश्रण के दो सेट को एससी और सीएफ सिस्टम में विभाजन की तुलना करने के लिए चुना गया था। ओवलबिमिन (45 केडीए, पीआई -4.0) और लाइसोसिम (14.5 केडीए, पीआई -11.4) मिश्रण का उपयोग 30 केडीए पॉलीएटरसफोफ़ोन झिल्ली (पीईएस 30) में आकार आधारित अंश का अध्ययन करने के लिए किया गया था। द्विआधारी मिश्रण के दूसरे सेट में, हीमोग्लोबिन (65 केडीए, पीआई -6.8) और समान आकार के प्रोटीन के ओवलबिन को 100 केडीए पॉलीएटरसफॉल्फ झिल्ली (पीईएस 100) के उपयोग से चार्ज अंतर के आधार पर अलग किया गया था। यूएफ मॉड्यूल दोनों में हाइड्रोडायनामिक और भौतिक-रासायनिक मापदंडों की व्यापक श्रेणी के लिए पवित्रता, चयनात्मकता, संचरण, प्रवाह और गंदगी का अध्ययन किया गया था। झिल्ली सतह पर प्रोटीन सोखना भी परमाणु बल माइक्रोस्कोपी (एएफएम) का उपयोग करके अध्ययन किया गया था।

प्रोटीन अंशांकन एससी और सीएफ सिस्टम में टीएमपी (30, 60, 90 और 120 केपीए) और झिल्ली कतरनी तनाव (0.215, 0.61, 1.715 और 3.15 पा) के विभिन्न हाइड्रोडायनामिक मापदंडों में अध्ययन किया गया था। आकार आधारित विभाजन में, लाइसोसिम (शुद्धता > 98%) का सबसे अच्छा विभाजन टीपीएम में 30 केपीए और यूएफ सिस्टम दोनों के लिए 1.715 पी के झिल्ली कतरनी में मिला था। शुद्धता टीएमपी और कतरनी की स्थिति का अध्ययन करने के लिए कम संवेदनशील थी। लाइसोसिम ट्रांसमिशन ~ 70% की वसूली के साथ 90% से अधिक था सभी ऑपरेटिंग हालत में, अनुसूचित जाति में प्रोटीन जुदाई को लगातार झिल्ली कतरनी की स्थिति के तहत सीएफ सिस्टम में सफलतापूर्वक पुनः पेश किया गया था। प्रभावी आधारित पृथक्करण के मामले में, झिल्ली फोर्गिंग का शुद्धता और प्रोटीन के संचरण पर महत्वपूर्ण प्रभाव पड़ा। हीमोग्लोबिन (~ 60%) की लगभग समान शुद्धता दोनों प्रणालियों में 30 केपीए और 0.215 पा कतरनी की स्थिति के टीएमपी में प्राप्त की गई थी। सबसे अच्छा विभाजन प्रोटीन विभाजन के दोनों तरीकों के लिए कम टीएमपी और कतरनी हालत में पाया गया था।

भौतिक-रासायनिक स्थिति जैसे पीएच और आयनिक ताकत का आकार आधारित प्रोटीन जुदाई के लिए शुद्धता पर बहुत कम प्रभाव था। ल्यूसोसिम ट्रांसमिशन, पीएच और ईओनिक शक्ति दोनों यूएफ सिस्टम में फ्रीड के साथ स्पष्ट रूप से भिन्न है। लाइसोसिम की उच्चतम शुद्धता > 95% 65% की वसूली के साथ प्राप्त की गई थी दूसरी ओर, पीएच और ईओण शक्ति का प्रभार आधारित प्रोटीन जुदाई में प्रोटीन चुनिंदा पर महत्वपूर्ण प्रभाव था। फ्रीड समाधान के पीएच को बदलकर प्रोटीन की चुनौती को उलट किया जा सकता है। प्रोटीन adsorbed झिल्ली के माध्यमिक sieving के कारण अनुसूचित जाति CF से बेहतर चयन था। उच्च शुद्धता (एचबी - 88% और ओवा - 83%) और उच्च वसूली (~ 70%) एससी मॉड्यूल में कम ईओण शक्ति पर प्राप्त की गई थी।

प्रोटीन विभाजन के दौरान समय के साथ फ्लक्स गिरावट एससी और सीएफ मॉड्यूल में विभिन्न हाइड्रोडायनामिक और भौतिक-रासायनिक अवस्था के लिए अध्ययन किया गया था। उच्च कतरनी और कम टीएमपी यूएफ मॉड्यूल दोनों में न्यूनतम प्रवाह में गिरावट रखने की सबसे अच्छी स्थिति है। आकार आधारित विभाजन की तुलना में प्रभारी आधारित विभाजन में फ्लक्स गिरावट अधिक थी। अनुसूचित जाति प्रवाह गतिशीलता में अंतर्निहित अंतर के कारण लगातार कतरनी हालत के तहत सीएफ के मुकाबले अधिक प्रवाह में गिरावट की घोषणा की। आयन की ताकत और फ्रीड समाधान का पीएच प्रवाह में गिरावट पर महत्वपूर्ण प्रभाव पड़ा। इलेक्ट्रोस्टैटिक इंटरैक्शन की अनुपस्थिति के कारण उच्च प्रवाह की कमी प्रोटीन की ऊपरी आयनिक शक्ति और आइसोइलेक्ट्रिक बिंदु पर पाया गया था। एससी झिल्ली पर स्थान आधारित प्रोटीन सोखना एएफएम सतह विश्लेषण के माध्यम से अध्ययन किया गया था। परिपत्र झिल्ली के केंद्रीय क्षेत्र में उच्च औसत सतह खुरदरापन (रा) माध्यमिक रेडियल आवक प्रवाह के कारण झिल्ली पर उच्च प्रोटीन सोखना की पुष्टि करता है।

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List of Symbols

- A_f - Filtration Area (m^2)
- C_b - Solute concentration in bulk (kgm^{-3})
- C_m - Solute concentration near membrane surface (kgm^{-3})
- C_P - Solute concentration in Permeate (kgm^{-3})
- C_R - Solute concentration in Retentate (kgm^{-3})
- d - Distance from membrane to centreline in cross flow system (L)
- D - Diffusive coefficient of solute (ms^{-1})
- D_t - Diameter of stirred cell (cm)
- D_i - Diameter of impeller (cm)
- E - Electrical potential (V)
- F - Force exerted by AFM cantilever (N)
- f - Frictional coefficient of protein (no unit)
- g_x, g_y, g_z - liquid velocity due to gravity in x, y, z direction (LT^{-1})
- J_v - Volumetric flux (ms^{-1})
- J_{ss} - pseudo-steady state flux (ms^{-1})
- J_w - Pure water flux (ms^{-1})
- L_p - Hydraulic permeability ($m^2kg^{-1}s^{-1}$)
- n - Number of impeller (no unit)
- r - Radial distance from centre in stirred cell unit (L)

R - Radius of stirred cell unit (cm)
 R_a - Average roughness of membrane (nm)
 R_m - Membrane resistance (m^{-1})
 R_p - Polarisation resistance (m^{-1})
 R_f - Fouling resistance (m^{-1})
 K - Spring constant in AFM (N/m)
 k - Mass transfer coefficient (ms^{-1})
 u - Radial velocity component (LT^{-1})
 V_p - Permeate volume (m^3)
 v - Circumferential velocity component (LT^{-1})
 $v_f(r)$ - Local filtration velocity normal to membrane (LT^{-1})
 V_x, V_y, V_z - Cross flow velocity of liquid in x, y, z direction (LT^{-1})
 w - Axial velocity component (LT^{-1})
 z - Distance at any point from membrane surface (L)
 Re - Reynolds number ($r\omega^2\rho/\mu$)
 Sc - Schmidt number ($\mu/\rho D$)
 Sh - Sherwood number (Kr/D)
TFA - Trifluoro acetic acid
 Q - Volumetric flow rate (L^3T^{-1})
 V_p - Permeate volume (m^{-3})

v_m, v_{avg} – Maximum velocity and average velocity of liquid across cross flow system ($L T^{-1}$)

x - Cantilever deflection in AFM (nm)

Z - Net charge of protein (no unit)

Greek Symbols

ρ - Density of feed ($kg m^{-3}$)

ϕ - Module geometry constant (dimensionless)

τ_o - Observed transmission coefficient (dimensionless)

τ - True transmission coefficient (dimensionless)

τ_{zr} - Shear stress due to radial velocity (Pa)

$\tau_{z\phi}$ - Shear stress due to circumferential velocity (Pa)

τ_w - Wall shear stress (Pa)

Ψ - Selectivity of protein (dimensionless)

ω - angular velocity (rad/s)

ΔP - Transmembrane pressure (Pa)

μ - Viscosity of feed ($kg m^{-1} s^{-1}$)

μ_e - Electrophoretic mobility ($m^2 s^{-1} V^{-1}$)

ξ - Dimensionless distance from membrane surface (no unit)

ν - Kinematic viscosity ($m^2 s^{-1}$)