Process for continuous purification of single-chain antibody fragments based on Simulated Moving Bed Chromatography

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Zusammenfassung

In den letzten Jahren ist der Markt für rekombinante Antikörperfragmente sehr schnell gewachsen und die Antiköperfragmente stellen im Bereich der Therapeutika eine neue Alternative zu den monoklonalen Antikörpern dar. Die sogenannten single-chain Fragmente (scFv), welche über aktive Antigenbindungsstellen verfügen, sind besonders attraktiv in Forschung, Diagnostik und Therapie. Aufgrund signifikanter Erfolge im Upstream-Verfahren besteht ein starkes Bedürfnis an effizienten kontinuierlichen Aufreinigungsprozessen, die rekombinante Proteine isolieren können, im Vergleich zu den etablierten mehrstufigen diskontinuierlichen chromatographischen Prozessen.

Diese Doktorarbeit beschreibt die theoretische Auslegung und experimentelle Validierung eines Simulated Moving Bed (SMB) Prozesses unter Verwendung der sogenannten Immobilized Metal ion Affinity Chromatography (IMAC) für die Aufreinigung eines single-chain Antikörperfragment. Ziel ist es, das Antikörperfragment aus dem Zellkulturüberstand in möglichst hoher Reinheit zu gewinnen. Zuerst wurde der Zellkulturüberstand mit dem Antikörperfragment mittels einer stufenweisen pH-Gradienten Batch-Chromatographie charakterisiert. Der Einfluss der Zusammensetzung des Lösungsmittels (pH) auf die Adsorptionsisothermen wurde durch pH-Gradienten Batch-Versuche untersucht.

Die vorgeschlagene Auslegung basiert auf der rekursiven Lösung eines Gleichgewichtsstufenmodells, welches einen entsprechenden True Moving Bed (TMB) Prozess beschreibt. Mögliche Betriebsbedingungen wurden theoretisch untersucht und experimentell im Labor-Maßstab SMB-Anlage mit einem methodischen Ansatz realisiert.

Die Ergebnisse des kontinuierlichen SMB Prozesses wurden mit Resultaten für das einfachere Batch-Verfahren hinsichtlich Reinheit, Ausbeute und Produktivität verglichen. Diese Arbeit liefert den experimentellen Nachweis der Machbarkeit dieses neuartigen SMB Prozesses für die kontinuierliche Aufreinigung von Antikörperfragmenten.

Abstract

During the last years the market for recombinant antibody fragments has been growing very fast. Small-sized fragments can offer new alternatives to the full-length monoclonal antibodies (mAb) in the field of antibody-based therapeutics. Among them, the single-chain antibody fragments (scFv) are the smallest antibody fragments that still possess a complete antigen-binding site. Hence, they are ideal for research, diagnostic and therapy, which require good tissue penetration. The high titers obtained with recombinant proteins are imposing a tremendous challenge in the development of more efficient continuous purification processes, which can isolate recombinant proteins in an easier way, compared to the established multi-step discontinuous chromatographic scheme.

This work focuses on the theoretical design and experimental validation of a Simulated Moving Bed (SMB) chromatography process using Immobilized Metal Ion Affinity Chromatography (IMAC) for the purification of a single-chain antibody fragment. First, the cell culture supernatant containing the antibody fragment and originating from Bacillus megaterium was characterized using a stepwise pH-gradient batch chromatography. The influence of solvent composition (pH) on the adsorption isotherm parameters of the antibody fragment and its impurities were determined in single-column runs.

Based on the estimated adsorption isotherm parameters a feasible multicolumn open-loop 3-zone pH-gradient SMB process is suggested, which possesses the potential to isolate continuously the single-chain antibody fragment. The design is based on a recursive solution of an equilibrium stage model of an equivalent True Moving Bed (TMB) process. Possible operating conditions were theoretically proposed using the simulations obtained using the model and experimentally realized in a lab-scale SMB unit using a methodological approach.

The observed performance of the continuous process is compared to the corresponding batch process in terms of purity, yield, productivity, and buffer consumption. The potential of this innovative SMB process is clearly demonstrated.

Nomenclature

Latin Symbols	Definition
A_c	Cross-sectional area of the column
c^k	Concentration of component k
D_{ax}^k	Axial Diffusion coefficient
F	Phase ratio
H^k	Adsorption equilibrium constant of k
k	Capacity factor
k'_0	Retention factor
L	Column lenght
m^k	Mass of component k
m_i	Dimensionless flow rate ratio in zone i
N_{eq}	Number of equilibrium stages
q^k	Loading of component k
$t_{injection}$	Injection time
t_R	Retention time
t_{switch}	Switching time
t_0	Hold-up time
t_{cycle}	Cycle time
u	Linear velocity
$V_{i,dead}$	Extra-column dead volumen in zone i
V_{SP}	Stationary phase volume
V_{MP}	Mobile phase volume

V_c	Column volume
$\dot{V}$	Flow rate
$\dot{V}_i^{SMB}$	Flow rate of zone i in SMB
$\dot{V}_i^{TMB}$	Flow rate of zone i in TMB
$\dot{V}_s$	Flow rate of stationary phase
w	Peak width
Y	Yield
x	Position coordinate

Greek Symbols	Definition
β	Safety factor
χ	Dimensionless factor chi
η	Dimensionless factor eta
γ	Dimensionless factor gamma
ε	Porosity
μ	First and second central moment

Subscripts and superscripts	Definition
D	Desorbent
E	Extract
F	Feed
$fraction$	Refered to collected fraction in batch
k	Component k
$port$	Refered to outlet port in SMB
R	Raffinate

Abbreviations	Definition
AC	Affinity Chromatography
B. megaterium	Bacillus megaterium

CHO	Chinese Hamster Ovary cells
CSS	Cyclic steady state
E. coli	Escherichia coli
ELISA	Enzyme-linked Immunosorbent Assay
Fab	Fragment antigen binding
FDA	US Food and Drug Administration
GMP	Good Manufacturing Practices
HETP	Height Equivalent to a Theoretical Plate
HIC	Hydrophobic Interaction Chromatography
IEC	Ion Exchange Chromatography
IgG	Immunoglobulin Gamma
IMAC	Immobilized Metal Ion Affinity Chromatography
mAbs	Monoclonal Antibodies
MOL	Method of lines
ODE	Ordinary differential equation
PDE	Partial differential equation
PUR	Purity
PRD	Specific productivity
scFv	single-chain antibody fragment
SDC	Specific buffer consumption
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel ele.
SEC	Size Exclusion Chromatography
SMB	Simulated Moving Bed Chromatography
TMB	True Moving Bed Chromatography

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