OPTIMIZATION OF PROCESS CHROMATOGRAPHY: MODELING AND EXPERIMENTAL APPROACHES

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Optimization of Process Chromatography:

Modeling and Experimental Approaches

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Certificate

This is to certify that the thesis entitled **"Optimization of Process Chromatography: Modeling and Experimental Approaches "** being submitted by **Vijesh Kumar** to the Indian Institute of Technology, Delhi, for the award of the degree of Doctor of Philosophy, is a record of bonafide research work carried out by him. **Vijesh Kumar** has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

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Abstract

One of the most significant contributors to the production cost of development and manufacturing of therapeutic proteins is downstream processing. Amongst the various downstream processing unit operations, chromatography is arguably the most critical with respect to the high selectivity it offers. However, chromatography presents a major process development challenge due to the large number of process parameters such as pH, ion concentration, gradient and stationary phase, that impact the performance of the step as well as quality of the resulting product. Models can also be judiciously used to reduce lab experimentation, thereby significantly reducing the time required and the cost incurred during development of a chromatography step. Two types of models have been developed in this work viz. empirical and mechanistic model. Cation exchange process has been modeled for removal of removal of product aggregates and charge variants. The first part of the thesis deals with development of empirical model based on design of experiments (DOE) and of novel methods obtaining optimal separation of product aggregates and charge variants. Through this work, we have compared the various DOE designs used in bioprocessing and proposed an optimal approach. To enhance the use of the various criteria for comparing competing models two case studies have been used. In the second part of the thesis, mechanistic models have been developed using general rate model and a novel extended Langmuir model. Calibration of model and parameter estimation were performed using inverse methods for accurate values. The model developed was demonstrated for prediction of charge variants of product in a wide range of operating conditions. The model was also successfully applied as a PAT tool for facilitating real-time peak pooling for separation of charge variants. In nutshell, this work has successfully demonstrated use of models for practical applications involving use of cation exchange chromatography for separation of product related impurities.

सार

चिकित्सीय प्रोटीन के विकास और निर्माण के उत्पादन लागत के लिए सबसे महत्वपूर्ण योगदानकर्ताओं में से एक डाउनस्ट्रीम प्रोसेसिंग है विभिन्न डाउनस्ट्रीम प्रोसेसिंग यूनिट ऑपरेशंस में, क्रोमैटोग्राफी यह उच्च चुनौती देने के संबंध में सबसे महत्वपूर्ण है, जो यह पेशकश करती है। हालांकि, क्रोमैटोग्राफ़ी प्रक्रिया प्रक्रियाओं जैसे पीएच, आयन एकाग्रता, ढाल और स्थिर चरण की बड़ी संख्या के कारण एक प्रमुख प्रक्रिया विकास चुनौती प्रस्तुत करती है, जो कि परिणाम के साथ-साथ उत्पाद के गुणवत्ता के साथ ही गुणवत्ता के प्रदर्शन को प्रभावित करती है। प्रयोगशाला प्रयोग को कम करने के लिए मॉडल्स का भी इस्तेमाल किया जा सकता है जिससे आवश्यक समय कम हो और क्रोमैटोग्राफी चरण के विकास के दौरान लागत कम हो। इस कार्य में दो प्रकार के मॉडल विकसित किए गए हैं अर्थात अनुभवजन्य और यंत्रवत् मॉडल । सेशन एक्सचेंज प्रक्रिया को उत्पाद समुच्चय और चार्ज वेरिएंट को हटाने के लिए तैयार किया गया है। थीसिस का पहला भाग प्रयोगों के डिजाइन (डीओई) के आधार पर एक अनुभवजन्य मॉडल के विकास के साथ-साथ उत्पाद समुच्चय और चार्ज वेरिएंट का इष्टतम विभाजन प्राप्त करने के तरीकों का भी उल्लेख करता है। इस काम के माध्यम से, हमने बायोप्रोसेसिंग में इस्तेमाल किए गए विभिन्न डीओई डिज़ाइनों की तुलना की है और एक इष्टतम दृष्टिकोण प्रस्तावित किया है। प्रतिस्पर्धा मॉडल की तुलना करने के लिए विभिन्न मानदंडों के उपयोग को बढ़ाने के लिए दो मामले अध्ययनों का इस्तेमाल किया गया है। थीसिस के दूसरे भाग में, सामान्य दर मॉडल और उपन्यास विस्तारित लैंगमुइर मॉडल का उपयोग करके यंत्रवत् मॉडल विकसित किए गए हैं। सटीक मूल्यों के लिए उलटा तरीकों का उपयोग करके मॉडल और पैरामीटर आकलन के अंशांकन का प्रदर्शन किया गया। विकसित मॉडल ऑपरेटिंग परिस्थितियों की एक विस्तृत श्रृंखला में उत्पाद के प्रभार प्रकार के पूर्वानुमान के लिए प्रदर्शित किया गया है। प्रभारी रूपों के अलग होने के लिए रीयल-टाइम पीक पूलिंग की सुविधा के लिए मॉडल को सफलतापूर्वक एक पैट टूल के रूप में लागू किया गया है। संक्षेप में, यह काम उत्पाद संबंधी

अशुद्धियों के पृथक्करण के लिए सेशन एक्सचेंज क्रोमैटोग्राफी के उपयोग से संबंधित व्यावहारिक अनुप्रयोगों के लिए मॉडल का सफलतापूर्वक उपयोग किया गया है।

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Abbreviations

A1	acidic 1
A2	acidic 2
AICc	Akaike information criterion corrected
ANOVA	analysis of variance
B1	basic 1
B2	basic 2
BB	Box-Behnken
BIC	Bayesian information criterion
CADET	chromatography analysis and design toolkit
CC	central composite
CEX	cation exchange chromatography
CHO	Chinese hamster ovary
Cov	covariance
CI	Capto impress SP
CPP	critical process parameters
CQA	critical quality attributes
CS	Ceramic hyperD S
CSTRs	continuous stirred tank reactors
CV	column volume
DBC	dynamic binding capacity
DO	D-optimal
DO16	D-optimal 16 runs
DOE	design of experiments
DPFRs	dispersed plug flow reactors
EXS	Eushmuno XS
FC	Fractogel COO^-
FF	full factorial
FS	Fractogel S
GCSF	granulocyte colony-stimulating factor
GRM	general rate model
H	Henry’s constant
HCP	host cell proteins
HEPES	4-2-hydroxyethyl-1-piperazineethanesulfonic acid
HETP	height equivalent to the theoretical plate
HIC	hydrophobic interaction chromatography

HMW	high molecular weight
HPLC	high performance liquid chromatography
HTPD	high-throughput process development
IEC	ion exchange chromatography
IO	I-optimal,
IO 14	I-optimal 14 runs
IO16	I-optimal 16 runs
M	main
mAbs	monoclonal antibodies
MVDA	multivariate data analysis
ODE	ordinary differential equation
OFAT	one factor at a time
PAT	process analytical technology
PCA	principle component analysis
PHS	Poros HS
PLS	partial least square
PRESS	predicted residual sums of squares
p-value	probabilty value of hypothesis testing in statistics
PXS	Poros XS
QbD	quality by design
R^2	coefficient of determination
RMSE	root mean square of errors
RP-HPLC	reverse phase high performance liquid chromatography
SEC	size exclusion chromatography
SE-HPLC	size exclusion high performance liquid chromatography
SMA	steric mass action
SS	sum of squares
UPLC	ultra-performance liquid chromatography
UV	ultraviolet
X	design matrix

Notations

α	emperical constant in henry's function
β	regression coefficients
$\hat{\beta}$	estimate of regression coefficients
cov	covariance
$c^{j}_{meas,i}$	measured concentrations at the ith time point of the jth experiment

c_{in}	inlet feed concentration
c_p	protein concentration in mobile phase
c_s	salt concentration in mobile phase
$\mathbf{c_{sim}\ p, t}$	simulated concentrations at the system outlet
D_{ax}	column axial dispersion coefficient
$\boldsymbol{D_p}$	pore diffusion coefficient
D_s	axial dispersion coefficient in DPFR external system volume
ε	error
$\boldsymbol{\varepsilon_c}$	column porosity
$\boldsymbol{\epsilon_p}$	particle porosity
k	equilibrium constant
k_p	association constant in extended Langmuir model
k_{film}	film diffusion coefficient
k_a	adsorption rate constant
$\boldsymbol{k_d}$	desorption rate constant
L	column length
$\mathbf{N_m}$	number of measurement sets/experiments
$\mathbf{N_{pj}}$	number of data points in the jth experiment
$\hat{\boldsymbol{p}}$	log-transformed parameters
$\boldsymbol{q_{max}}$	maximum adsorption capacity on solid phase
q_p	protein concentration in mobile phase
q_s	salt concentration in solid phase
u	interstitial column velocity
$\boldsymbol{r}$	radial coordinate
Λ	ionic capacity
σ	steric factor
σ^2	variance
$\boldsymbol{resp}$	least squares residual
$\boldsymbol{r_p}$	bead radius
R^2	coefficient of determination
τ	mixing time of CSTR's
$\mathbf{t_i^j}$	ith time point of the jth experiment
ν	characteristic charge
X	design matrix
$\boldsymbol{z}$	axial coordinate