

PROCESS ANALYTICAL TECHNOLOGY APPLICATIONS FOR HARVEST UNIT OPERATIONS

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By

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Submitted

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To



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Dedicated to my family...

न हि ज्ञानेन सदृशं पवित्रमिह विद्यते ।
तत्स्वयं योगसंसिद्धः कालेनात्मनि विन्दति

*In this world, there is nothing as purifying as knowledge. One who
has attained purity of mind through prolonged practice of Yog,
receives such knowledge within the heart, in due course of time.*

Bhagavad Gita: Chapter 4, Verse 38

CERTIFICATE

This is to certify that the thesis entitled “**PROCESS ANALYTICAL TECHNOLOGY APPLICATIONS FOR HARVEST UNIT OPERATIONS**”, being submitted by **SHANTANU BANERJEE** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy**, is a record of the original bonafide research work carried out by him under our guidance and supervision. 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.

We certify that he has pursued the prescribed course of research.

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SHANTANU BANERJEE

Abstract

In today's technological era, smart factories within manufacturing industries are not a distant prospect. Industrial sectors are shifting toward digitalization and automation as a precondition for Industry 4.0. In spite of its remarkable rise, which saw it reach a \$188 billion sales value in 2017, the biopharmaceutical industry clearly trails behind in this shift. Data analytics and digitalisation have been recognized as crucial strategic initiatives for enabling continuous manufacturing and faster and cheaper biotherapeutic production. Initiatives such as Quality-by-design (QbD) and Process Analytical Technology (PAT) are considered white knights of the biopharmaceutical industry for ensuring consistent product quality in a complex manufacturing process and the key towards process automation. This thesis is centred around the application of PAT in the Chinese hamster ovary (CHO) cell harvest bioprocessing area using CAS, Pall Lifesciences, USA. The study involves rapid monitoring of key process parameters (KPPs) using online turbidity sensors and employing mechanistic, empirical and hybrid modelling approaches to achieve real-time monitoring, control, optimization and integration of the unit operation in continuous biopharmaceutical manufacturing.

The first work (Chapter 3) involves KPP identification in CAS, which affects the two primary process outputs: cell separation efficiency (CSE) and protein recovery (PR). A mechanistic model based on the population balance model (PBM) along with an acoustic agglomeration kernel was developed to predict cell size distribution as a function of time, resulting in CSE estimation. While an empirical model using the design of experiments (DoE) approach was used to screen out KPP affecting PR. Results show that $> 90\%$ CSE was achieved for feed flowrate (FF) $< 5 \text{ mL min}^{-1}$ and acoustic power (AP) $> 12.5 \text{ W}$ while $> 90\%$ PR was obtained for FF $> 15 \text{ mL min}^{-1}$. Both the models combined formed a hybrid model, and experimental results validate its accuracy in predicting the CHO agglomeration process. Furthermore, a generalized correlation has been developed using the Buckingham Pi theorem between the dimensionless numbers composed of relevant processes and geometrical variables to predict the agglomeration efficiency. To our knowledge, this is the first such study to model the agglomeration dynamics of CHO cells in acoustic standing waves.

The next study (Chapter 4) proposes a Digital twin (DT) and a cyber-physical system (CPS) for CAS. While the CPS - DT serves as the core, a real-time model based on the developed hybrid models has been implemented in tandem to present a highly reliable hybrid model to improve the model's adaptability. The framework employs a distributed control system (DCS), the heart of the CPS architecture, that updates the parameters of the physical system in real-

time, thereby simplifying process control. To evaluate the hybrid-model predictive control strategy in real time, three case studies were conducted, which involved introducing abrupt turbidity pulses of high, low, and multi values. The sudden deviation in the turbidity of the feed sample was controlled by adjusting the AP of the chamber (the control variable). The use of the proposed controller resulted in a 5 % mean deviation from the setpoint in the first chamber, while a > 33 % mean deviation resulted in the absence of the controller. The study's outcome demonstrates the high effectiveness of hybrid model-based CPS - DT control in consistently achieving high CSE (> 90 %) and integrating the unit operation in a continuous processing train to establish a smart manufacturing prototype for the biopharmaceutical industry.

Following development of CPS architecture, the next study (Chapter 5) introduces a methodology for developing a holistic smart manufacturing framework comprising 4 interactive modules. These modules deal with process control, prognosis, and diagnosis and act as an external communication platform using different methodologies. The architecture, real-time monitoring, controller experiments, and detailed discussions are presented to implement the automatic process control (APC) algorithm on CAS. The CPS framework was designed to address the aforementioned objectives and was enhanced with deep learning-based neural networks at various levels. The proposed CPS controller resulted in a 5% mean deviation from the setpoint in the first chamber, while more than 33% resulted in the controller's absence. In addition, the time taken by the framework to monitor and implement control action is 10 s, leading to robust process regulation even in case of a minor deviation. In the absence of the CPS framework, the monitoring becomes purely manual and is prone to significant process deviation. If a disturbance occurs, the error can propagate downstream due to delayed implementation of corrective action. It is envisaged that the proposed CPS framework powered by ML models can provide solutions for a compendious ‘smart’ approach for real-time process monitoring and control of all unit operations in the manufacturing industry.

Finally, after the development of a comprehensive framework for monitoring, multi-objective optimization was performed on the acoustic clarification using CAS (Chapter 6). Three objectives, namely CSE, PR and residence time (RT) of harvest, were evaluated using a hybrid model approach. Next, the non-dominated sorting genetic algorithm (NSGA-II) was successfully employed for tri-objective optimization using KPPs, namely FF, CF and load cell density (LCD) as the decision variables. The optimization study was performed for three different configurations of the acoustic chambers: series, series–parallel, and parallel to obtain the optimal design and set of solutions. The results of the NSGA-II algorithm revealed the

relationships between CSE, PR, and RT and evaluated a tradeoff and an optimal balance between them was achieved. The series–parallel configuration was evaluated as the most optimal design, giving CSE: 80.20 %, PR: 82.61 % and RT: 10.42 mins at an LCD of 30×10^6 cells mL^{-1} . An application study was conducted to obtain the optimal solution set at different LCDs in a single run. Further, controller studies were also performed to apply corrective actions in case of feed turbidity disturbances in order to maintain a steady state. Overall, this is the first attempt to develop a multi-objective optimization study for CHO cell clarification, and this may be utilized as a tool for simulation, design, optimization, and control of any cell harvest unit operation.

सारांश

आज के तकनीकी युग में, विनिर्माण उद्योगों के भीतर स्मार्ट कारखाने एक दूर की संभावना नहीं हैं। औद्योगिक क्षेत्र उद्योग 4.0 की पूर्व शर्त के रूप में डिजिटलीकरण और स्वचालन की ओर बढ़ रहे हैं। अपनी उल्लेखनीय वृद्धि के बावजूद, जिसने देखा कि यह 2017 में 188 बिलियन डॉलर के बिक्री मूल्य तक पहुंच गया, बायोफार्मास्युटिकल उद्योग स्पष्ट रूप से इस बदलाव में पीछे है। डेटा विश्लेषण और डिजिटलीकरण को निरंतर विनिर्माण और तेज और सस्ते जैव चिकित्सा उत्पादन को सक्षम करने के लिए महत्वपूर्ण रणनीतिक पहलों के रूप में मान्यता दी गई है। क्वालिटी-बाय-डिजाइन (QbD) और प्रोसेस एनालिटिकल टेक्नोलॉजी (PAT) जैसी पहलों को एक जटिल विनिर्माण प्रक्रिया में लगातार उत्पाद की गुणवत्ता सुनिश्चित करने और प्रक्रिया स्वचालन की कुंजी के लिए बायोफार्मास्युटिकल उद्योग का सफेद शूरवीर माना जाता है। यह शोध प्रबंध कैडेन्स एकाॅस्टिक सेपरेटर (CAS), पाल लाइफसाइंसेज, USA का उपयोग करके चीनी हम्सटर अंडाशय (CHO) कोशिका फसल जैव प्रसंस्करण क्षेत्र में पीएटी के अनुप्रयोग के आसपास केंद्रित है। अध्ययन में ऑनलाइन टर्बिडिटी सेंसर का उपयोग करके प्रमुख प्रक्रिया मापदंडों (KPP) की तेजी से निगरानी और निरंतर बायोफार्मास्युटिकल विनिर्माण में इकाई संचालन की वास्तविक समय की निगरानी, नियंत्रण, अनुकूलन और एकीकरण प्राप्त करने के लिए यांत्रिक, अनुभवजन्य और संकर मॉडलिंग दृष्टिकोण को नियोजित करना शामिल है।

पहले कार्य (अध्याय 3) में CAS में KPP पहचान शामिल है, जो दो प्राथमिक प्रक्रिया आउटपुट को प्रभावित करता है: कोशिका पृथक्करण दक्षता (CSE) और प्रोटीन रिकवरी (PR) एक ध्वनिक समूह कर्नेल के साथ जनसंख्या संतुलन मॉडल (PBM) पर आधारित एक यांत्रिक मॉडल को समय के एक कार्य के रूप में कोशिका आकार वितरण की भविष्यवाणी करने के लिए विकसित किया गया था, जिसके परिणामस्वरूप CSE अनुमान लगाया गया था। जबकि प्रयोगों के डिजाइन (DoE) दृष्टिकोण का उपयोग करते हुए एक अनुभवजन्य मॉडल का उपयोग PR को प्रभावित करने वाले KPP की जांच करने के लिए किया गया था। परिणाम बताते हैं कि फ्रीड फ्लो रेट (FF) $< 5 \text{ mL min}^{-1}$ और ध्वनिक शक्ति (AP) $> 12.5 \text{ W}$ के लिए $> 90\%$ CSE हासिल किया गया था, जबकि $> 90\%$ PR FF $> 15 \text{ mL min}^{-1}$ के लिए प्राप्त किया गया था। दोनों मॉडलों ने संयुक्त रूप से एक संकर मॉडल का निर्माण किया, और प्रयोगात्मक परिणाम CHO एकत्रीकरण प्रक्रिया की भविष्यवाणी करने में इसकी सटीकता को मान्य करते हैं। इसके अलावा, एकत्रीकरण दक्षता की भविष्यवाणी करने के लिए प्रासंगिक प्रक्रियाओं और ज्यामितीय चर से बनी आयामहीन संख्याओं के बीच बर्किंघम पाई प्रमेय का उपयोग करके एक सामान्यीकृत सहसंबंध विकसित किया गया है। जहाँ तक हमारी जानकारी है, ध्वनिक स्थायी तरंगों में CHO कोशिकाओं के एकत्रीकरण गतिकी को मॉडल करने वाला यह पहला अध्ययन है।

अगला अध्ययन (अध्याय 4) CAS के लिए एक डिजिटल ट्विन (DT) और एक साइबर-फिजिकल सिस्टम (CPS) का प्रस्ताव करता है। जबकि CPS-DT कोर के रूप में कार्य करता है, मॉडल की अनुकूलन क्षमता में सुधार के लिए एक अत्यधिक विश्वसनीय हाइब्रिड मॉडल प्रस्तुत करने के लिए विकसित हाइब्रिड मॉडल पर आधारित एक वास्तविक समय मॉडल को लागू किया गया है। फ्रेमवर्क सीपीएस आर्किटेक्चर के केंद्र में एक वितरित नियंत्रण प्रणाली (DCS) को नियोजित करता है, जो वास्तविक समय में भौतिक प्रणाली के मापदंडों को अपडेट करता है, जिससे प्रक्रिया नियंत्रण सरल हो जाता है। वास्तविक समय में संकर-मॉडल भविष्यसूचक नियंत्रण रणनीति का मूल्यांकन करने के लिए, तीन मामले अध्ययन किए गए, जिनमें उच्च, निम्न और बहु मूल्यों की अचानक टर्बिडिटी दालों को पेश करना शामिल था। फ्रीड नमूने की अशांति में अचानक विचलन को कक्ष के एपी (नियंत्रण चर) को समायोजित करके नियंत्रित किया गया था प्रस्तावित नियंत्रक के उपयोग के परिणामस्वरूप पहले कक्ष में सेटपॉइंट से 5% माध्य विचलन हुआ, जबकि $> 33\%$ माध्य विचलन के परिणामस्वरूप नियंत्रक की अनुपस्थिति हुई। अध्ययन के परिणाम उच्च CSE ($> 90\%$)

को लगातार प्राप्त करने और बायोफार्मास्युटिकल उद्योग के लिए एक स्मार्ट विनिर्माण प्रोटोटाइप स्थापित करने के लिए एक निरंतर प्रसंस्करण ट्रेन में इकाई संचालन को एकीकृत करने में हाइब्रिड मॉडल-आधारित CPS-DT नियंत्रण की उच्च प्रभावशीलता को प्रदर्शित करते हैं।

CPS वास्तुकला के विकास के बाद, अगला अध्ययन (अध्याय 5) एक समग्र स्मार्ट विनिर्माण ढांचे को विकसित करने के लिए एक कार्यप्रणाली का परिचय देता है जिसमें 4 इंटरैक्टिव मॉड्यूल शामिल हैं। ये मॉड्यूल प्रक्रिया नियंत्रण, पूर्वानुमान और निदान से संबंधित हैं और विभिन्न पद्धतियों का उपयोग करके एक बाहरी संचार मंच के रूप में कार्य करते हैं। CAS पर स्वचालित प्रक्रिया नियंत्रण (APC) एल्गोरिदम को लागू करने के लिए वास्तुकला, वास्तविक समय की निगरानी, नियंत्रक प्रयोग और विस्तृत चर्चा प्रस्तुत की जाती है। सी. पी. एस. ढांचे को उपरोक्त उद्देश्यों को पूरा करने के लिए डिज़ाइन किया गया था और विभिन्न स्तरों पर गहन शिक्षण-आधारित तंत्रिका नेटवर्क के साथ बढ़ाया गया था। प्रस्तावित सीपीएस नियंत्रक के परिणामस्वरूप पहले कक्ष में सेटपॉइंट से 5% माध्य विचलन हुआ, जबकि 33% से अधिक नियंत्रक की अनुपस्थिति में हुआ। इसके अलावा, नियंत्रण कार्रवाई की निगरानी और कार्यान्वयन के लिए ढांचे द्वारा लिया गया समय 10s है, जिससे मामूली विचलन के मामले में भी मजबूत प्रक्रिया विनियमन होता है। सी. पी. एस. ढांचे की अनुपस्थिति में, निगरानी विशुद्ध रूप से मैनुअल हो जाती है और महत्वपूर्ण प्रक्रिया विचलन के लिए प्रवण होती है। यदि कोई गड़बड़ी होती है, तो सुधारात्मक कार्रवाई के कार्यान्वयन में देरी के कारण त्रुटि नीचे की ओर फैल सकती है। यह परिकल्पना की गई है कि एम. एल. मॉडल द्वारा संचालित प्रस्तावित CPS ढांचा विनिर्माण उद्योग में सभी इकाई संचालनों की वास्तविक समय प्रक्रिया निगरानी और नियंत्रण के लिए एक व्यापक 'स्मार्ट' दृष्टिकोण के लिए समाधान प्रदान कर सकता है।

अंत में, निगरानी के लिए एक व्यापक ढांचे के विकास के बाद, CAS (अध्याय 6) का उपयोग करके ध्वनिक स्पष्टीकरण पर बहु-उद्देश्य अनुकूलन किया गया था तीन उद्देश्यों, अर्थात् सी. एस. ई., पी. आर. और फसल के निवास समय (RT) का मूल्यांकन एक संकर मॉडल दृष्टिकोण का उपयोग करके किया गया था। इसके बाद, गैर-प्रधान छँटाई आनुवंशिक एल्गोरिथम (NSGA-II) को निर्णय चर के रूप में KPP, अर्थात् एफएफ, सीएफ और लोड सेल डेंसिटी (LCD) का उपयोग करके त्रि-उद्देश्य अनुकूलन के लिए सफलतापूर्वक नियोजित किया गया था। अनुकूलन अध्ययन ध्वनिक कक्षों के तीन अलग-अलग विन्यासों के लिए किया गया था: श्रृंखला, श्रृंखला-समानांतर, और इष्टतम डिज़ाइन और समाधानों के सेट को प्राप्त करने के लिए समानांतर। NSGA-II एल्गोरिथम के परिणामों ने CSE, PR और RT के बीच संबंधों का खुलासा किया और एक समझौते का मूल्यांकन किया और उनके बीच एक इष्टतम संतुलन हासिल किया गया। श्रृंखला-समानांतर विन्यास का मूल्यांकन सबसे इष्टतम डिज़ाइन के रूप में किया गया था, जिसमें सीएसई: 80.20%, पीआर: 82.61% और आरटी: 10.42 मिनट 30×10^6 cells mL⁻¹ के LCD पर दिए गए थे। एक ही दौड़ में विभिन्न LCD पर इष्टतम समाधान सेट प्राप्त करने के लिए एक अनुप्रयोग अध्ययन आयोजित किया गया था। इसके अलावा, एक स्थिर स्थिति बनाए रखने के लिए फीड टर्बिडिटी गड़बड़ी के मामले में सुधारात्मक कार्यों को लागू करने के लिए नियंत्रक अध्ययन भी किए गए थे। कुल मिलाकर, यह CHO कोशिका स्पष्टीकरण के लिए एक बहु-उद्देश्य अनुकूलन अध्ययन विकसित करने का पहला प्रयास है, और इसका उपयोग किसी भी कोशिका फसल इकाई संचालन के अनुकरण, डिज़ाइन, अनुकूलन और नियंत्रण के लिए एक उपकरण के रूप में किया जा सकता है।

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List of Symbols

- A_{ij} : agglomeration kernel involving particles of size x_i and x_j ($\text{m}^3 \text{s}^{-1}$)
- c : speed of sound (m s^{-1})
- F_{acx}, F_{acy} and F_{acz} : acoustic force in the three directions (kg m s^{-2})
- F_{dx}, F_{dy} and F_{dz} : drag force in the three directions (kg m s^{-2})
- $F_{g'}$: net gravitational force (gravity-buoyancy) (kg m s^{-2})
- g : acceleration of gravity ($\text{m}^{-1} \text{s}^2$)
- I : total number of sections covering entire size range
- K : bulk modulus of medium (kg m s^{-2})
- K_p : bulk modulus of particle (kg m s^{-2})
- K_r : reflection coefficient of reflector
- k : magnitude of wave vector (m^{-1})
- k_x, k_y and k_z : wave vector in x, y and z direction (m^{-1})
- L : length of the chamber (m)
- $n(v,t)dv$: number of particles in the size range v to $v+dv$, at time t (m^{-3})
- $n_f dv$: number of particles in the size range v to $v+dv$, in the feed (m^{-3})
- N_f : total number of particles in the i th size range, in the feed per unit volume of slurry (m^{-3})
- $N_i(t)$: total number of particles in the i th size range, at time t per unit volume of slurry (m^{-3})
- p : acoustic pressure ($\text{kg m}^{-1} \text{s}^{-2}$)
- Q : feed flow rate ($\text{m}^3 \text{s}^{-1}$)
- Q_s : volumetric flow rate of particle settling ($\text{m}^3 \text{s}^{-1}$)
- r_p : radius of particle (m)
- ratio : a parameter that defines the geometric grid of the type $x_{i+1} = \text{ratio} * x_i$
- $\tilde{u}_z$: mechanical displacement amplitude (m)
- U_{px}, U_{py} and U_{pz} : particle velocity in the three directions (m s^{-1})
- U_f : velocity due to the flow field (m s^{-1})
- V_{12}^0 : relative velocity between two particles (m s^{-1})
- V_t : system volume (volume of the region of acoustic wave) (m^3)

v : volume of particles (m^3)

v_i, v_{i+1} : lower and upper boundaries for i^{th} section (m^3)

x_i : representative volume for the i^{th} size range, also the i^{th} grid point (m^3)

x_{Is} : critical settling volume (m^3)

y_c^* : critical impact parameter (m)

Greek letters

β_p : compressibility of particle ($\text{kg}^{-1} \text{m}^{-1} \text{s}^2$)

β : compressibility of fluid ($\text{kg}^{-1} \text{m}^{-1} \text{s}^2$)

φ : acoustic contrast factor

Ψ_0 and Ψ_1 : scattering coefficients (m^3)

ρ : fluid density (kg m^{-3})

ρ_p : particle density (kg m^{-3})

η_s : separation efficiency

μ_f : viscosity of the fluid ($\text{kg m}^{-1} \text{s}^{-1}$)

$\Gamma(v_1, v_2)$: agglomeration rate for a pair of particles with volumes v_1 and v_2 ($\text{m}^3 \text{s}^{-1}$)

ω : angular frequency (s^{-1})

ω_p : resonant frequency of particle (s^{-1})

List of Abbreviations

AICc	Akaike information criterion
ANOVA	Analysis of variance
AP	Acoustic power
APC	Automatic process control
ATF	Alternating tangential flow
AWS	Acoustic wave separation
BIC	Bayesian information criterion
CAS	Cadence TM acoustic separator
CCB	Cell culture broth
CF	Concentrate flowrate
CHO	Chinese hamster ovary
CIP	Cleaning-in-place
CPS	Cyber-physical system
CQA	Critical quality attribute
CSE	Cell separation efficiency
CSPR	Cell-specific perfusion rate
DCS	Distributed control system
DE	Differential evolution
DNN	Deep neural network
DoE	Design of experiments
DSP	Downstream processing
DT	Digital twin
EMA	European medical agency
FF	Feed flowrate
FTIR	Fourier-transform infrared spectroscopy
HCCF	Harvested cell culture fluid
HCDNA	Host cell deoxyribonucleic acid
HCP	Host cell protein
KPP	Key process parameter
LCD	Load cell density
mAb	Monoclonal antibody
MAE	Mean absolute error

MAPE	Mean absolute percentage error
MSE	Mean square error
NIR	Near-infrared spectroscopy
NRMSE	Normalised root mean square error
NSGA-II	Non-dominated sorting genetic algorithm
NTU	Nephelometric turbidity unit
PBM	Population balance model
PCC	Periodic counter-current chromatography
PID	Partial integro-differential
PLC	Programmable logic controller
PLS	Partial least squares
PR	Protein recovery
PRESS	Predicted error sum of squares
PSO	Particle swarm optimization
QbD	Quality by design
R^2	Coefficient of determination
RF	Random forest
RMSE	Root mean square error
RSM	Response surface methodology
RT	Residence time
RTD	Residence time distribution
RTRT	Real-time release testing
SCADA	Supervisory control and data acquisition
SIP	Sterilization-in-place
SPC	Statistical process control
SPTFF	Single-pass tangential flow ultrafiltration
SVR	Support vector regression
TFF	Tangential flow filtration
USFDA	United States Food and Drug Administration
USP	Upstream processing
VCD	Viable cell density