

MODELING OF LIQUID CHROMATOGRAPHY

EMPIRICAL AND MECHANISTIC MODELING APPROACHES

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MODELING OF LIQUID CHROMATOGRAPHY

EMPIRICAL AND MECHANISTIC MODELING APPROACHES

by

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.....Dedicated to my elder sister

Kiran Kanwar

CERTIFICATE

This is to certify that the thesis entitled “**MODELING OF LIQUID CHROMATOGRAPHY: EMPIRICAL AND MECHANISTIC MODELING APPROACHES**” being submitted by **LALITA KANWAR SHEKHAWAT** 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 her under my 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. I certify that she has pursued the prescribed course of research.

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ABSTRACT

Major global regulatory bodies such as the US Food and Drug Administration (FDA) have been encouraging biopharmaceutical manufacturers to adopt the recent initiatives of quality by design (QbD) in order to improve robustness of biotech processes and consistency in quality of the resulting product. With the advent of QbD, model assisted process development and optimization is gaining importance in the biopharmaceutical industry. Models assist in a more efficient, systematic, and economical process development as they can efficiently investigate design alternatives with minimal experimentation and in deriving complete process understanding. Further, process analytical technology (PAT) which covers the process control aspects of QbD, is gradually being implemented to achieve real time process control and consistency in the quality of the final product. Different modeling approaches that have applied in the biopharmaceutical industry can be classified into empirical models that are based on design of experiments (DOE) and mechanistic models that are based on the underlying fundamental processes of the system. Major expectations from a good model as desired by industry include accurate prediction and feasibility with respect to implementation in an industrial environment. This thesis documents modeling of different modes of chromatography including Protein-A affinity chromatography, ion-exchange chromatography, ion-exchange membrane chromatography, hydrophobic interaction chromatography using empirical and mechanistic modeling based approaches.

Under empirical modeling cation exchange chromatography has been modeled for separation of charge variants and aggregates and hydrophobic interaction chromatography for removal of aggregates. Empirical modeling involved two major challenges, one being a large number of process factors resulting in large number of experiments and another being large number of output response variables making model evaluation difficult. In view of these challenges, we propose a split DOE approach for reducing the number of experiments and for simplification of model evaluation.

Further, Protein-A resin fouling has been empirically modeled. Protein-A resins constitute the largest expense in downstream processing. Therefore, economic feasibility demands that the resin be reused for 50 to 300 cycles before being discarded. Resin reuse is, however, accompanied by resin fouling caused by deposition of foulants on the exterior and the interior surfaces of the resin. Fouling impacts both binding as well as mass transfer characteristics of the resin. We have proposed an empirical model for reliable prediction of performance of

Protein-A resin as well as for improved understanding of the underlying fouling mechanism responsible for decline in resin performance during fouling.

Next mechanistic modeling of ion-exchange membrane chromatography has been performed using a simplified form of general rate model obtained through the eliminations of film and pore diffusions for prediction of breakthrough capacity of membrane adsorber. Selection of appropriate adsorption isotherm to identify operating conditions that would result in high binding capacities and appropriate binding kinetics in order to understand binding mechanisms was also performed.

Finally mechanistic model for hydrophobic interaction chromatography (HIC) has been performed using general rate model coupled with exponentially modified Langmuir adsorption model to predict elution profiles of main mAb product and aggregate impurity. The developed HIC mechanistic model has been applied as a PAT tool for making robust pooling decisions to enable clearance of aggregates for a monoclonal antibody (mAb) therapeutic.

सार

अमेरिका के खाद्य और औषधि प्रशासन (एफडीए) जैसी प्रमुख वैश्विक नियामक संस्थाएं बायोपार्मिक निर्माताओं को बायोटेक प्रक्रियाओं की मजबूती और परिणामी उत्पाद की निरंतरता में सुधार के लिए डिजाइन (क्यूबीडी) द्वारा गुणवत्ता की हालिया पहल को अपनाने के लिए प्रोत्साहित कर रही हैं। QbD के आगमन के साथ, मॉडल सहायता प्राप्त प्रक्रिया विकास और अनुकूलन बायोफार्मास्यूटिकल उद्योग में महत्व प्राप्त कर रहा है। मॉडल अधिक कुशल, व्यवस्थित और किफायती प्रक्रिया विकास में सहायता करते हैं क्योंकि वे न्यूनतम प्रयोग के साथ डिजाइन विकल्पों की कुशलता से जांच कर सकते हैं और पूरी प्रक्रिया को समझने में मदद कर सकते हैं। इसके अलावा, प्रक्रिया विश्लेषणात्मक तकनीक (PAT) जो QbD के प्रक्रिया नियंत्रण पहलुओं को कवर करती है, को अंतिम उत्पाद की गुणवत्ता में वास्तविक समय प्रक्रिया नियंत्रण और स्थिरता प्राप्त करने के लिए धीरे-धीरे लागू किया जा रहा है। बायोफार्मास्यूटिकल उद्योग में लागू होने वाले विभिन्न मॉडलिंग दृष्टिकोणों को अनुभवजन्य मॉडल में वर्गीकृत किया जा सकता है जो प्रयोगों के डिजाइन (डीओई) और यंत्रवत मॉडल पर आधारित होते हैं जो सिस्टम की अंतर्निहित मूलभूत प्रक्रियाओं पर आधारित होते हैं। उद्योग द्वारा वांछित एक अच्छे मॉडल की प्रमुख अपेक्षाओं में औद्योगिक वातावरण में कार्यान्वयन के संबंध में सटीक भविष्यवाणी और व्यवहार्यता शामिल है। यह थीसिस प्रोटीन-ए आत्मीयता क्रोमैटोग्राफी, आयन-एक्सचेंज क्रोमैटोग्राफी, आयन-एक्सचेंज झिल्ली क्रोमैटोग्राफी, हाइड्रोफोबिक इंटरैक्शन क्रोमैटोग्राफी सहित अनुभवजन्य और यांत्रिकी मॉडलिंग आधारित दृष्टिकोणों का उपयोग करके क्रोमैटोग्राफी के विभिन्न तरीकों का मॉडलिंग करती है। अनुभवजन्य मॉडलिंग के तहत कटियन एक्सचेंज क्रोमैटोग्राफी को चार्ज वेरिएंट और एग्रीगेट के पृथक्करण और एग्रीगेट्स को हटाने के लिए हाइड्रोफोबिक इंटरैक्शन क्रोमैटोग्राफी के लिए तैयार किया गया है। अनुभवजन्य मॉडलिंग में दो प्रमुख चुनौतियां शामिल थीं, एक बड़ी संख्या में प्रक्रिया कारक थे जिसके परिणामस्वरूप बड़ी संख्या में प्रयोग हुए और एक और बड़ी संख्या में आउटपुट प्रतिक्रिया चर थे, जो मॉडल मूल्यांकन को कठिन बना रहे थे। इन चुनौतियों के मद्देनजर, हम प्रयोगों की संख्या को कम करने और मॉडल मूल्यांकन के सरलीकरण के लिए एक विभाजित डीओई दृष्टिकोण का प्रस्ताव करते हैं। इसके अलावा, प्रोटीन-ए राल फाउलिंग को अनुभवजन्य रूप से तैयार किया गया है। प्रोटीन-ए रेजिन डाउनस्ट्रीम प्रसंस्करण में सबसे बड़ा खर्च होता है। इसलिए, आर्थिक व्यवहार्यता मांग करती है कि राल को त्यागने से पहले 50 से 300 चक्रों के लिए पुनः उपयोग किया जाए। राल पुनः उपयोग किया जाता है, हालांकि, बाहरी और राल के आंतरिक सतहों पर फाउलेंट्स के जमाव के कारण राल फाउलिंग के साथ होता है। फाउलिंग दोनों बाध्यकारी के साथ-साथ राल के बड़े पैमाने पर स्थानांतरण विशेषताओं को प्रभावित करता है। हमने प्रोटीन-ए रेजिन के प्रदर्शन की विश्वसनीय भविष्यवाणी के लिए एक अनुभवजन्य मॉडल का प्रस्ताव दिया है और साथ ही साथ फाउलिंग के दौरान राल के प्रदर्शन में गिरावट के लिए जिम्मेदार अंतर्निहित

फाउलिंग तंत्र की बेहतर समझ के लिए। आयन-एक्सचेंज झिल्ली क्रोमैटोग्राफी का अगला मैकेनिस्टिक मॉडलिंग झिल्ली adsorber की सफलता क्षमता की भविष्यवाणी के लिए फिल्म और ताकना विवर्तन के माध्यम से प्राप्त सामान्य दर मॉडल के सरलीकृत रूप का उपयोग करके किया गया है। परिचालन स्थितियों की पहचान करने के लिए उपयुक्त सोखना इज़ोटेर्म का चयन जो बाध्यकारी प्रक्रियाओं को समझने के लिए उच्च बाध्यकारी क्षमताओं और उचित बाइंडिंग कैनेटीक्स में परिणाम होगा।

अंत में हाइड्रोफोबिक इंटरैक्शन क्रोमैटोग्राफी (एचआईसी) के लिए यंत्रवत मॉडल को मुख्य mAb उत्पाद और कुल अशुद्धता के क्षालन प्रोफाइल की भविष्यवाणी करने के लिए घातीय रूप से संशोधित Langmuir सोखना मॉडल के साथ मिलकर सामान्य दर मॉडल का उपयोग करके प्रदर्शन किया गया है। विकसित एचआईसी यांत्रिकी मॉडल को एक मोनोक्लोनल एंटीबॉडी (एमएबी) चिकित्सीय के लिए समुच्चय की मंजूरी के लिए मजबूत पूर्ण निर्णय लेने के लिए एक पैट उपकरण के रूप में लागू किया गया है।

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