FOULING OF RESIN IN PROTEIN A CHROMATOGRAPHY-MECHANISM, MONITORING AND CONTROL

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FOULING OF RESIN IN PROTEIN A CHROMATOGRAPHY-MECHANISM, MONITORING AND CONTROL

by

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.....Dedicated
to my
Family

CERTIFICATE

This is to certify that the thesis entitled "**Fouling of Resin in Protein A Chromatography-Mechanism, Monitoring and Control**" being submitted by **Ms. MILI VASANTKUMAR PATHAK** 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 her. MILI VASANTKUMAR PATHAK 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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…….Mili Vasantkumar Pathak

ABSTRACT

Understanding the mechanism of protein A chromatography fouling remains the center of interest for the manufacturers of biotherapeutics in order to increase resin lifetime. To understand the current practices carried out by manufacturers, a survey was performed jointly by researchers at Indian Institute of Technology Delhi and University College London. The survey targeted a total of 15 major manufacturers of mAb products, including five Indian manufacturers. During resin reuse various types of foulants can accumulate on Protein A resins as a result of non-specific, irreversible interaction of either the product or media. Binding and mass transport properties of widely used agarose-based Protein A resin, MabSelect SuRe^{TM}, have been examined to understand the mechanism of resin fouling. There could be various factors that impact resin fouling. These include product/impurity build-up due to components in the feed material and ligand degradation due to the use of harsh buffers. To unravel their contributions, cycling studies were performed with and without product loading. The results presented in this paper provide a lucid understanding of the causative factors that limit protein A chromatographic resin lifetime. The capacity fell for protein A resin at the end of 100th cycle due to use of feed material was found to be five times greater than that without using feed material. Compared to the fresh resin, the cycled resin samples showed 24% reduction in particle porosity and 51% reduction in pore mass transfer coefficient. Transmission electron microscopy (TEM) was used to qualitatively monitor accumulation of foulants on the cycled resin. Fouled resin sample contained a dense residue in the interior and exterior of resin particle both as a film at the bead surface and as granules. The surface activation energy increased five times in the case of fouled resin sample. The major event in fouling was identified as the non-specific adsorption of the feed material components on resin, signaling that pore diffusion is the rate limiting step. To provide a thorough understanding of fouling of Protein A resin due to feed material components,

cycling studies were carried out using three different feed materials (H1, H2, and H3) with varying feed components like proteases, histones, DNA, non-histone proteins. The IgG loading and the processing steps were kept similar in all the three cases. Changes in the process and the quality attributes were measured from yield, dynamic binding capacity at 10% of breakthrough curve, inter and intra-particle porosity, proteases in elute, protein A leachate, aggregate and histone content in the elute. The yield and DBC were found to be similar in all the three cases but there was significant decrease observed in particle porosity.

Resin reuse is accompanied by two major events, one is the loss of protein A ligands due to leaching or inactivation and other is fouling due to non-specific, irreversible interactions between the resin particles and the product/ other foulants. This thesis presents, a novel, fluorescence based approach towards direct, in-situ measurement of protein A ligand density as well as monitoring of resin fouling during resin reuse. The proposed approach has been proven to be robust (error <1%) and can be effectively used for developing of cleaning and sanitization protocols during process development as well as for monitoring and control of fouling so as to gain longevity in resin life. The proposed approach has been successfully used for development of a two-step cleaning protocol that has been shown to be significantly more effective than the conventionally used cleaning regimens both with respect to observed loss of protein A ligand, dynamic binding capacity, as well as product yield. Application of this protocol resulted in the dynamic binding capacity at 93% of the original value at 50 cycles (vs. 73% with conventional protocols) and > 90% at 200 cycles. This study would be of great interest to manufacturers of monoclonal antibody based therapeutic products.

सार

प्रोटीन की व्यवस्था को समझना क्रोमैटोग्राफी दूषण रेजिन जीवनकाल को बढ़ाने के लिए बायोथेरेप्यूटिक्स के निर्माताओं के लिए ब्याज का केंद्र बना रहता है। निर्माताओं द्वारा की गई मौजूदा प्रथाओं को समझने के लिए, इंडियन इंस्टीट्यूट ऑफ टेक्नोलॉजी दिल्ली और यूनिवर्सिटी कॉलेज लंदन के शोधकर्ताओं द्वारा संयुक्त रूप से एक सर्वेक्षण किया गया। इस सर्वे ने एमएबी उत्पादों के कुल 15 प्रमुख निर्माताओं को लक्षित किया, जिनमें पांच भारतीय निर्माताओं शामिल थे। राल के पुन: उपयोग के दौरान विभिन्न प्रकार के फौलेट्स उत्पाद या मीडिया के गैर-विशिष्ट, अपरिवर्तनीय संपर्क के परिणामस्वरूप प्रोटीन ए रेजिन पर जमा कर सकते हैं। व्यापक रूप से प्रयुक्त एगरोज़-आधारित प्रोटीन ए राल के बाध्यकारी और बड़े पैमाने पर परिवहन गुणों, रासिन दूषण के तंत्र को समझने के लिए जांच की गई है। विभिन्न कारक हो सकते हैं जो प्रभाव राल दूषण को प्रभावित करते हैं। इसमें कड़े बफ़र्स के उपयोग के कारण फ़ीड सामग्री और लीगैंड डिग्रेडेशन में घटकों के कारण उत्पाद / अशुद्धता बिल्ड-अप शामिल हैं अपने योगदान को सुलझाने के लिए, बिना उत्पाद लोड होने के साथ, साइकिल चालन के अध्ययन किए गए थे। इस पत्र में प्रस्तुत परिणाम कारक कारकों की एक समझदार समझ प्रदान करते हैं जो प्रोटीन ए क्रोमैटोग्राफिक राल जीवनकाल को सीमित करते हैं। क्षमता प्रोटीन के लिए गिर गई फ़ीड सामग्री के उपयोग के कारण 100 वें चक्र के अंत में एक राल पाया गया था कि फ़ीड सामग्री का उपयोग किए बिना पांच गुना अधिक है। ताजा राल की तुलना में, साइकिल वाले राल के नमूनों में कण के छिद्रों में 24% की कमी और ताकिक द्रव्यमान हस्तांतरण गुणांक में 51% कमी दिखाई गई। ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी (टीईएम) का उपयोग गुणात्मक रूप से साइक्लिक राल पर फौलेटों के संचय पर निगरानी रखने के लिए किया गया था। फूल्ड राल नमूना में मनका सतह पर एक फिल्म के रूप में राण कण के इंटीरियर और बाहरी दोनों में घने अवशेष शामिल थे और कणिकाओं के रूप में। फॉल्ड राल नमूने के मामले में सतह सक्रियण ऊर्जा में पांच गुना वृद्धि हुई। दूषण में प्रमुख घटना को राल पर फ़ीड सामग्री घटकों के गैर-विशिष्ट सोखना के रूप में पहचाना गया था, यह संकेत है कि ताकना प्रसार दर सीमित कदम है। खाद्य सामग्री घटकों के कारण प्रोटीन ए राल के दूषण की संपूर्ण समझ प्रदान करने के लिए, साइकिलिंग अध्ययनों में तीन अलग-अलग फ़ीड सामग्री (एच 1, एच 2 और एच 3) का उपयोग किया गया था, जैसे कि प्रोटीस, हिस्टोन, डीएनए, गैर

हिस्टोन प्रोटीन । आईजीजी लोडिंग और प्रसंस्करण कदम सभी तीन मामलों में समान रखा गया था। प्रक्रिया में परिवर्तन और गुणवत्ता के गुणों को उपज, गतिशील बाध्यकारी क्षमता से 10% सफलता चक्र, अंतर और इंट्रा-कण छिद्रण, नमूनों में प्रोटीन, प्रोटीन लीलेट, समेकित और हिस्टोन सामग्री को एल्यूट में मापा गया। उपज और डीबीसी सभी तीन मामलों में समान पाए गए थे, लेकिन कणों की छिद्र में बहुत कम कमी देखी गई थी।

राल का दोबारा प्रयोग दो प्रमुख घटनाओं के साथ होता है, एक प्रोटीन का नुकसान leaching या निष्क्रियता के कारण एक ligands और अन्य गैर-विशिष्ट, रेजिन कणों और उत्पाद / अन्य अशुद्धियों के बीच रिवर्सिव इंटरैक्शन के कारण दूषित है। यह थीसिस प्रस्तुत करता है, एक उपन्यास, प्रतिदीप्ति पुन: उपयोग के दौरान प्रोटीन ए लिगैंड घनत्व के साथ-साथ राल फोगिंग की निगरानी के प्रत्यक्ष, अंतर-माप के प्रति प्रतिदीप्ति आधारित दृष्टिकोण प्रस्तुत करता है। प्रस्तावित दृष्टिकोण मजबूत (त्रुटि <1%) साबित हुआ है और प्रक्रिया विकास के दौरान सफाई और स्वच्छता प्रोटोकॉल के विकास के लिए प्रभावी ढंग से इस्तेमाल किया जा सकता है, साथ ही साथ दूषण नियंत्रण और नियंत्रण के लिए ताकि राल जीवन में दीर्घायु हासिल कर सके। प्रस्तावित दृष्टिकोण का दो-चरण सफाई प्रोटोकॉल के विकास के लिए सफलतापूर्वक उपयोग किया गया है जो पारंपरिक रूप से इस्तेमाल किए जाने वाले सफाई के नियमों से काफी अधिक प्रभावी साबित हुआ है, प्रोटीन ए लिगैंड, गतिशील बाध्यकारी क्षमता, साथ ही उत्पाद प्राप्ति। इस प्रोटोकॉल का प्रयोग 50 चक्रों पर मूल मूल्य के 93% पर गतिशील बाध्यकारी क्षमता (पारंपरिक प्रोटोकॉल के साथ 73%) और 200 चक्रों पर> 90% पर हुआ। यह अध्ययन मोनोक्लोनल एंटीबॉडी आधारित चिकित्सीय उत्पादों के निर्माताओं के लिए बहुत रुचि का होगा।

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