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Adaptation of the pore diffusion model to describe multi-addition batch uptake high-throughput screening experiments

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ABSTRACT

Equilibrium isotherm and kinetic mass transfer measurements are critical to mechanistic modeling of binding and elution behavior within a chromatographic column. However, traditional methods of measuring these parameters are impractically time- and labor-intensive. While advances in high-throughput robotic liquid handling systems have created time and labor-saving methods of performing kinetic and equilibrium measurements of proteins on chromatographic resins in a 96-well plate format, these techniques continue to be limited by physical constraints on protein addition, incubation and separation times; the available concentration of protein stocks and process pools; and practical constraints on resin and fluid volumes in the 96-well format. In this study, a novel technique for measuring protein uptake kinetics (multi-addition batch uptake) has been developed to address some of these limitations during high-throughput batch uptake kinetic measurements. This technique uses sequential additions of protein stock to chromatographic resin in a 96-well plate and the subsequent removal of each addition by centrifugation or vacuum separation. The pore diffusion model was adapted here to model multi-addition batch uptake and was tested and compared with traditional batch uptake measurements of uptake of an Fc-fusion protein on an anion exchange resin. Acceptable agreement between the two techniques is achieved for the two solution conditions investigated here. In addition, a sensitivity analysis of the model to the physical inputs is presented and the advantages and limitations of the multi-addition batch uptake technique are explored.

1. Introduction

Chromatography remains the workhorse for protein purification across the biopharmaceutical industry [1]. As such, model-based process characterization is desirable to improve process understanding and robustness [2–4]. To this end, descriptions of protein equilibrium isotherms and intra-particle protein transport are critical to achieve accurate modeling of chromatographic column behavior [5]. The time required to obtain such experimental measurements has limited further exploration and implementation of rigorous process models in the biopharmaceutical industry. Batch uptake has traditionally been performed in an agitated vessel containing protein and chromatographic resin, where protein concentration in the fluid phase is measured and plotted over a period of hours as a function of elapsed time [5–7]. Modern high-throughput techniques hold significant promise to reduce both the time and effort of performing these measurements, while simultaneously increasing the experimental throughput.

Previous resin-based high-throughput studies have generally utilized a liquid-handling robot as a screening tool to estimate binding, wash, and elution conditions for chromatography step development [3,8–19], as summarized in detail in recent work [12,20,21]. While more rigorous studies have been performed looking at equilibrium isotherm [4,22–25] and batch uptake measurement [26], these have been limited in scope especially with regard to uptake kinetics and generally focused on adapting high-throughput systems to perform traditional measurements. This work explores a novel high-throughput workflow that is designed to provide detailed mechanistic information, while still taking advantage of the capabilities of the liquid handling robot.

While traditional methods for determining equilibrium isotherms and uptake kinetics can generally handle any volume of protein stock and resin slurry necessary to achieve the desired loading of protein on the resin [5], high-throughput experiments performed in micro-plates are generally performed within a range of 10–50 μL resin and 200–400 μL of protein stock. In cases where

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the load material is only available at low protein concentration (e.g. bioreactor harvest), adapting traditional batch experiments to the high-throughput format may be impractical. As protein stock concentration may affect the estimated intra-particle mass transfer parameter [27], it is highly desirable to perform these measurements under practically-relevant process conditions. The proposed technique, referred to as multi-addition batch uptake, aims to address this challenge by allowing multiple loading steps and further promises to rapidly provide intra-particle transport information over a variety of conditions in a single high-throughput experiment. Specifically, modification of the standard pore diffusion model is proposed and implemented to fit pore diffusivities following a similar approach to the more traditional batch uptake experiment [5].

While the multi-addition experimental technique has been successfully utilized to achieve target loading densities for investigation of elution behavior [28,29], the focus of this work is to quantify the uptake kinetics of the successive protein addition steps. Experimental methods will be described in greater detail in a separate work [30]. Here the objective is to mathematically define an appropriate model and validate it using multi-addition batch uptake and traditional batch uptake performed for the same protein and resin under similar solution conditions. In addition, special attention is paid here to the sensitivity of the fitted data to the experimental design and variability.

The objective of this work is to improve both the speed and ease of performing batch uptake measurements and to integrate this high-throughput robotic workflow to provide intra-particle uptake kinetic measurements for chromatographic modeling. These achievements would further contribute to a more robust process understanding and substantial reduction in experimental burden and material requirement during process development.

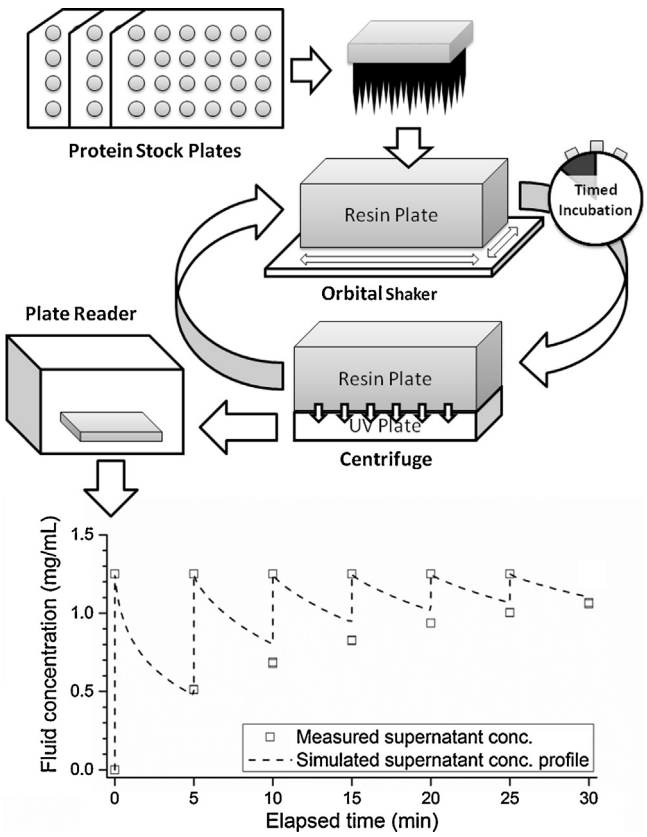

Fig. 1. Flow diagram of the multi-addition batch uptake experiment. Concentration measurements at the end of each timed (T = 5 min) incubation step are shown along with a simulated protein concentration profile as a function of elapsed time since the first resin contact with protein. Elapsed time during centrifugation and re-addition of protein to the plate are neglected. The initial protein concentration (C_0) for each incubation step shown here is 1.25 g/L.

2. Experimental

2.1. Materials

Buffer species and salts were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of an Fc-fusion protein (acidic pI) that had been purified through all necessary chromatography steps were prepared in 10 mM sodium phosphate at pH 7.0 with 0 and 50 mM sodium chloride. Chromatography resin (Q Sepharose Fast Flow) was obtained from GE Healthcare Life Sciences (Piscataway, NJ, USA). Resin stocks were charged with 1 M sodium chloride and then equilibrated with the appropriate equilibration buffer (0 mM and 50 mM sodium chloride at pH 7.0 in 10 mM sodium phosphate) and carefully settled in a graduated cylinder to achieve 50% v/v resin slurry.

2.2. Isotherm and batch uptake

Protein isotherms were measured using 0.1 mL pre-equilibrated resin slurry and 1.5 mL protein solution of desired concentration in microcentrifuge tubes of nominal capacity 1.5 mL. The sample was mixed overnight on a rotator and spun down using a benchtop centrifuge for 10 min at 3000 rpm, after which the final protein concentration in solution was measured by UV absorbance [31] using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Traditional batch uptake experiments were performed using an ÄKTA^{TM} avant 150 system (GE Healthcare Life Sciences, Piscataway, NJ, USA) connected to circulate protein stock within a stirred vessel containing 85.8 mL protein solution and 2 mL resin slurry [32].

2.3. Multi-addition batch uptake

Multi-addition batch uptake experiments were performed using a Tecan Freedom EVO 200 (Tecan US, Inc., Morrisville, NC, USA) equipped with a BioShake 3000 elm (Q. Instruments GmbH, Jena, DEU) and a Rotanta 46RSC Robotic centrifuge (Hettich America, L.P., Buford, GA, USA). The Tecan robot was then used to prepare multiple 96-well protein plates by diluting the protein stock solutions to the desired concentration in each well.

The resin plate was prepared by dispensing identical volumes of the equilibrated resin slurry into each well of a Pall AcroPrep 96-well 1 mL Filter Plate (Pall Corporation, Port Washington, NY, USA) with a 0.22 μm Supor membrane. The 96-channel Tecan MCA (Multi-Channel Arm) was used to transfer protein from a single stock plate to the resin plate as described in Fig. 1. Agitation was performed at 1000 rpm on the Bioshake for a pre-defined duration, after which the resin plate was transferred to the centrifuge. Supernatant with remaining protein was centrifuged from the resin plate into a deep-well collection plate at 1500 rpm for 1 min and saved for UV measurement.

Subsequent protein additions were performed identically to the first. Following the final protein addition and separation, the resin plate was washed with equilibration buffer and the bound protein eluted in 1 M sodium chloride. Elution protein concentrations were measured by UV to confirm the mass balance.

Further background and detail on the high-throughput experimental aspects of these measurements will be explored in a separate submission [30].