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Robust high-throughput batch screening method in 384-well format with optical in-line resin quantification

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ABSTRACT

High-throughput batch screening technologies have become an important tool in downstream process development. Although continuative miniaturization saves time and sample consumption, there is yet no screening process described in the 384-well microplate format. Several processes are established in the 96-well dimension to investigate protein–adsorbent interactions, utilizing between 6.8 and 50 μL resin per well. However, as sample consumption scales with resin volumes and throughput scales with experiments per microplate, they are limited in costs and saved time. In this work, a new method for in-well resin quantification by optical means, applicable in the 384-well format, and resin volumes as small as 0.1 μL is introduced. A HTS batch isotherm process is described, utilizing this new method in combination with optical sample volume quantification for screening of isotherm parameters in 384-well microplates. Results are qualified by confidence bounds determined by bootstrap analysis and a comprehensive Monte Carlo study of error propagation. This new approach opens the door to a variety of screening processes in the 384-well format on HTS stations, higher quality screening data and an increase in throughput.

1. Introduction

High-throughput screening (HTS) techniques in downstream process development have established new approaches and lead to significant advances in productivity and time to optimized processes. High-throughput batch screening methods are an important tool in early stage development of chromatography based purification strategies, due to their advantage in throughput. Batch chromatography experiments in HTS scale gain further importance with the development of computational models for large scale chromatography column runs, relying on protein–adsorbent interaction parameters [1–4]. Different batch screening technologies have been developed in recent years. Resin volumes distributed to filter plates have been used to screen preferable binding and elution conditions for biomolecules [5–7]. Hermann et al. developed a method to prepare equally sized resin plaques [8] which have been used in batch screening experiments [9]. Commercially available filter plates with pre-packed chromatographic materials were used to determine dynamic binding capacities [10]. Wenger et al. used pipetting tips filled with resin to purify virus-like particles in an HTS application [11].

Despite different approaches and ongoing advances in the development of HTS hardware, the 96-well format has remained the limit in throughput and sample size.

Data quality remains the challenge in HTS process development, as processing uncertainties gain significance with decreasing volumes. With increasing process complexity, single step error propagation gains impact on data quality. Process downscale is limited by the volumes of sample solutions and resins which can be handled reproducible. At the same time, sample consumption per experiment often scales with the resin volume utilized, resulting in high sample consumption for complex design spaces to be screened, despite the HTS approach.

If it is possible to overcome the limitation of erroneous, miniature volume by quantification rather than accurate volume handling, the range of 384-well would be made accessible for applications in process development. Therefore, in this work we solved the quantification problem via light extinction measurements in the sub-100 μL volume range. Optical measurements can be used to quantify volumes as well as suspensions of particles. Methods to quantify volumes in microtiter plates by vertical beam photometers utilizing the absorbance of the solvent water have been described and utilized in extent, confirming reliability [12,13].

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Particles can also be quantified, based on concentration and particle size distribution as they absorb and scatter light. In the limitation of small concentrations, the scattering of light by particles follows the Lambert–Beer law, as light beams impinge on single particles, they will most probably be represented as single spots on a sensor plate [14]. At higher concentrations particle–particle interactions occur. The linear correlation of particle concentration and light extinction does not hold true as with higher particle concentrations radiation undergoes interaction with multiple particles. Light scattered by one particle will hit a second one and so on, leading to an increase in transmission, compared to the Lambert–Beer law. According to the hard core model, particles at high concentrations will also expose more surface to radiation as they do not interpenetrate each other. This results in a decrease in radiation transmitted to the sensor plane [15,16,14].

Despite the HT approach, experimental data are limited, therefore measurements of statistical validity for reproducibility and fits of mechanistic models should be provided for such screening processes. Re-sampling techniques, as bootstrapping and Monte Carlo analysis, can be used to assess the distribution of experimental data points, and parameters derived, as well as to investigate error propagation in complex processes. Bootstrapping, a random re-sampling method, and non-parametric statistical techniques in general can be used to analyze data without assuming a particular probability distribution. Those distribution-free methods can be applied to a wide variety of statistical problems and do not require extensive assumptions on data distributions to validate analysis. The reasoning behind and an extensive description of this statistical method can be found in detail in [17–19].

Monte Carlo simulations allow for in silico calculation of process errors, given the description of the process in mechanistic equations and single process step uncertainties being quantified. This allows to analyze the influence of single process steps and their associated uncertainties on the overall experimental results. In-detail explanation of Monte Carlo techniques can be found in literature, e.g. [20–22]. Despite their advantages, examples of statistical validation of HTS results in literature are sparse. Kurup et al. describe a Monte Carlo error estimation in simulated moving bed chromatography [23]. Osberghaus et al. evaluated the error propagation in an HTS isotherm process, but limited the investigation to the effects on single measurement points, rather than isotherm parameters estimated [24].

In this work, signal extinction due to particle light scattering is utilized to accurately quantify volumes of adsorbent resins, distributed to microtiter plates. The applicability of this technique is shown for resins of different particle sizes and backbone composition. This new approach in chromatographic resin quantification is utilized in a newly developed automated batch isotherm HTS process in the 384-well format. The automated batch screening process presented here features an optical quantification of resin and volumes pipetted into a 384-well microtiter plate and yields 384 measurement points in a run time of approximately 4 h. Dependent on sample layout, this equals 12 (32 data points each) to 24 (16 data points) measured isotherms in one process cycle. Isotherm parameter estimations are evaluated by a bootstrap re-sampling method. Process uncertainties are quantified and their impact on parameter estimation is assessed by Monte Carlo simulation.

2. Materials and methods

2.1. Materials

Lysozyme from chicken egg white was purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium phosphate, sodium hydroxide and sodium chloride were purchased from Merck KGaA (Darmstadt, Germany). Strong cation-exchange adsorbent SP Sepharose FF was purchased from GE Healthcare (Buckinghamshire, United Kingdom), adsorbents Toyopearl SP 650M and SP 650C were acquired from Tosoh Bioscience GmbH (Stuttgart, Germany). Microtiter plates UVStar 96-well and UVStar 384-well (both plane bottom F-shape) were obtained from Greiner Bio-One GmbH (Frickenhausen, Germany).

2.2. Equipment

Resin plaques of defined volume of 7.8 μL and 20.8 μL, respectively, were produced with a ResiQuot device from Atoll-Bio (Weingarten, Germany) according to instructions from [8]. The batch process was automated on a Tecan Freedom Evo 200 robotic workstation (Tecan, Maennedorf, Switzerland). Main features of this station is a liquid handling arm (LiHa) consisting of eight separately controllable pipetting channels, each equipped with a fixed Teflon coated pipetting tip and driven by a 1 mL dilutor. A gripper (RoMa) was used for plate transfer on the workstation. Pipetting in 384-well plates was performed by consecutive use of a 96 channel pipetting head (MCA96) which was equipped with disposable tips of 200 μL volume. An integrated Hettich Rotanta 46RSC centrifuge (Hettich GmbH, Tuttlingen, Germany) was used for centrifugation of microtiter plates. An infinite 200M spectrophotometer (Tecan, Maennedorf, Switzerland) was utilized for optical measurements. Microtiter plates and disposable tips were stored in two storage units (Te-Stack) at the workstation and transferred to the worktable as needed. Pipetted liquid volumes were quantified with an analytical scale X S250 from Mettler-Toledo (Greifensee, Switzerland) which was integrated in the workstation.

Software EVOware 2.5 was used to program automated workflows on the liquid-handling workstation. The spectrophotometer was controlled by the Magellan 7.1 software, allowing for predefinition of measurement wavelengths and positions in well. Data evaluation and Monte Carlo simulations were performed in Matlab 8.0 (Mathworks, Natick, ME, USA).

2.3. Resin quantification

Samples in microtiter plates were centrifuged for 1 min at 2000 rpm prior to optical measurement to ensure even meniscus and full sedimentation of resin particles. Light extinction due to light scattered by adsorbent particles was measured at 330 nm. A grid of 6 by 6 evenly distributed measurement points with a distance of 50 μm to the well wall were measured in each well as shown in Fig. 2. Measurement values were averaged to account for uneven distribution of sedimented resin beads in the well.

Extinction coefficients for adsorbent materials were determined by dilution series in 384-well plates. Resin plaques of defined volumes were prepared in 96-well plates by use of the ResiQuot device and suspended in 300 μL buffer solution. Volumes of suspended resins were transferred to 384-well plates by pipetting, ensuring even distribution of adsorbent beads in suspension.

2.4. Batch binding process

The initial distribution of resin to a 96-well plate was performed manually utilizing the ResiQuot device. Resin plaques of 7.8 μL volume for Toyopearl SP 650C and SP 650M and 20.8 μL for Sepharose adsorbent were distributed in a 96-well plate as described in [8]. Plaques were dissolved in 300 μL 10 mM sodium phosphate buffer at pH 7.0, with varying sodium chloride concentrations, according to subsequent binding conditions. Protein stock solutions of 7.0 mg mL^{-1} concentration were prepared in the same buffer.