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Short communication

Measuring adsorption equilibria: The determination of the maximum binding capacity depends strongly on the method of resin preparation

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ABSTRACT

Accurate determination of adsorption isotherms is critical for designing chromatographic separations and selecting resins based on maximum binding capacity for the target molecule. This study evaluates different methods for preparing chromatography resins for batch adsorption isotherm experiments, comparing a volumetric slurry-based method, weight-based methods using vacuum filtration and centrifugation, and a commercial PreDictor^{TM} Isotherm plate to capacities observed in column operation.

The results show that weighing of hydrated particles after centrifugation, combined with density measurement provides the most accurate and reproducible binding capacity values, closely matching breakthrough data. This study highlights the importance of resin preparation methods in batch adsorption experiments and recommends the centrifugation method for non-automated workflows, as it provides the most reliable prediction of maximum binding capacity observed in column experiments. These findings are consistent for both anion- and cation-exchange resins with different resin backbones. This suggests the broad applicability of the proposed approach.

1. Introduction

Measuring adsorption isotherms is often the first step in designing chromatographic separation. They are used for initial resin selection based on capacity or selectivity depending on buffer conditions. High-throughput techniques with microtiter plates [1–4], robocolumns [5,6], resin filled pipette tips [7,8] and microfluidic chips [9,10] are applied to speed up and standardize this workflow and reduce sample consumption. Previous studies show that error margins for high throughput process development (HTPD) are related to the precise determination of resin amount used for the assay [11,12].

Most of these high throughput methods rely on dispensing the resin amount via a defined slurry concentration. Barker et al. [12] suggest to improve the accuracy by relating the slurry concentration to the actual amount of stationary phase dry mass contained in the slurry.

The utilization of high-throughput methods is predominantly comparative in nature, with the primary objective being the selection of resins that exhibit the highest binding capacity. If no HTPD equipment is available, the screening process can be performed by manual manipulation of resins and samples either in microtiter plates, or in small reaction tubes.

Once a resin has been selected and more sample is available, the most commonly used isotherm assay method is based on aliquoting a resin suspension. However, it suffers from the same inaccuracies as the HTPD methods. In our experience, the binding capacities obtained from the isotherm measurements often do not align with those obtained from column breakthrough experiments

In this study, we investigate different approaches to preparing chromatography resin starting material for isotherm experiments. We compare the standard volumetric slurry-based method to weight-based methods, with two different protocols for removing extra-particle liquid: vacuum filtration and centrifugation. Additionally, we compare our protocols with a commercial pre-dispensed PreDictor^{TM} Isotherm plate to match the obtained binding capacity values using the same stock solutions of protein to our methods. Finally, the batch data are then checked against capacity measurements performed in column operation mode.

In this study, Lysozyme on SP Sepharose FF and green fluorescent protein (GFP) on Toyopearl Gigacap Q650M are chosen as anion- and cation exchange model systems, respectively. We aim to show the differences between methods of determining adsorption isotherms and give a recommendation on the best protocol for performing batch adsorption experiments.

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2. Material and methods

2.1. Materials

The stationary phases used in this study were SP Sepharose FF purchased from Cytiva and Toyopearl Gigacap Q650M from Tosoh Bioscience, respectively. Model proteins were chicken egg white lysozyme obtained from Merck and green fluorescent protein (GFP), which was produced by an in-house purification process to a purity of > 99% as analyzed by HPLC SEC analysis. TRIS base, sodium acetate, sodium hydroxide and hydrochloric acid for buffer preparation were purchased from Merck Millipore.

2.2. Methods

2.2.1. Resin preparation and batch adsorption isotherms

2.2.1.1. Centrifugation method. Resins were washed 5 times in the respective equilibration buffer: 50mM sodium acetate, pH 5.0 for lysozyme on SP Sepharose FF and 10mM Tris, pH 8.5 for GFP on Gigacap Q650M.

The slurry was transferred to an Amicon^{TM} Ultrafree-CL Centrifugal filter device (Merck Millipore) with 5.0 μm pore size and centrifuged at 4000 g for 4 minutes. The density of the resulting hydrated particles was determined with a 25 mL pycnometer and found to be 1.04 g/cm^3 for SP Sepharose FF and 1.07 g/cm^3 for Gigacap Q650M.

2.2.1.2. Vacuum filtration method. Resins were washed 5 times in the respective equilibration buffer. The slurry was placed into a ceramic filter (pore size: 10 μm) on a vacuum flask. At a vacuum of 100 mbar the valve was opened and filtration was started. Filtration was stopped at discrete time points of 15, 30, and 60 seconds.

2.2.1.3. Slurry-based method. Resins were washed 5 times in equilibration buffer, settled overnight and a 50% (v/v) slurry was prepared.

2.2.1.4. Isotherm measurement. For all resin preparation methods, resins were weighed or pipetted into a 5 mL reaction tube to obtain a series of increasing phase ratios and were added to 3 mL of a 2 g/L protein solution. The suspensions were incubated overnight on an end-over-end rotator. Protein concentration in the supernatant was measured at 280 nm using a Cary 60 UV/Vis spectrophotometer (Agilent). Adsorbed protein was calculated by mass balance, and the resulting isotherm data were fitted to the Langmuir adsorption model to obtain the maximum binding capacity (q_{max}).

2.2.2. PreDictor^{TM} plate

SP Sepharose FF Isotherm plates were purchased from Cytiva. Four operators performed the assay according to manufacturer instruction with 2 g / L lysozyme solution in 50mM sodium acetate buffer pH 5.5. Absorption was measured with a Tecan plate reader (Tecan Life Sciences) at 280 nm. Adsorbed protein was calculated by mass balance and the resulting isotherm was established as described above.

2.2.3. Breakthrough curves

Breakthrough curves (BTC) were carried out on 2.5 mL prepacked columns from Repligen (L: 5 cm, i.d.: 0.8 cm) on an Äkta Pure 25 system from Cytiva. For accurate measurement of binding capacity, the void fraction was determined by non-binding pDNA pulse injections for Toyopearl Gigacap Q650M and Q Sepharose FF ($\varepsilon = 0.363$ and 0.343 respectively). The columns were supplied a 2 g / L solution of the respective model protein at 4 minutes residence time (75 cm / h). The effluent was monitored by absorbance at 280nm with the built-in UV monitor of the Äkta system. The resulting BTCs were fitted with a constant pattern solution for shrinking core behavior [13] to determine the maximum binding capacity.

2.2.4. Bed compression experiment

SP Sepharose FF resin was prepared with the centrifugal method and 5 grams were transferred to a Tricorn 10 (Cytiva) chromatography column. Packing buffer was added until the column was full. The resulting slurry was mixed and settled overnight. The column height was 10.4 cm which translates to a column volume of 8.2 mL (indicating a void fraction of ~0.4 in the slurry). The same resin amount was then flow packed to a stable bed height according to manufacturer's instructions. The compressed bed height was 9.5 cm (Volume: 7.76 mL) which is equivalent to a compression factor of ~1.09, which is in line with typical values for similar resins.

3. Results and discussion

Fig. 1 shows breakthrough curves of Lysozyme on SP Sepharose FF (Fig 1 a) and GFP on Toyopearl Gigacap Q650M (Fig.1b). The maximum binding capacity (q_{max}) values were obtained by fitting the experimental profiles with a column model based on the shrinking core model for pore diffusion [13], yielding 174 mg / mL particle for Lysozyme on SP Sepharose FF and 380 mg / mL particle for GFP on Gigacap Q650M. These values are in line with previously published maximum binding capacities [14,15]. These values were the benchmark against which the isotherm methods were evaluated.

Fig. 2 shows isotherms for Lysozyme and GFP obtained by pipetting a slurry suspension as outlined in the method section. Both isotherms are highly favorable and nearly rectangular. These isotherms were used to select a phase ratio suitable to perform adsorption assays to compare different methods for resin preparation. We chose data points in the middle of the plateau which are practically identical to the q_{max} value (red diamonds Fig. 2). Experiments were carried out at the chosen phase ratio in duplicates for all methods.

Additionally, we wanted to compare the different resin preparation methods to a standardized assay with given resin amounts. Consequently, we purchased an Isotherm PreDictor^{TM} plate of SP Sepharose FF from Cytiva. The assay was performed by four different operators according to manufacturer instructions and results are shown in Fig. 3. Obviously, there is a distinct trend of increasing capacities at higher phase ratios. This trend by far exceeds the expected increase of capacity as predicted by Langmuir adsorption behavior. As previously reported by Field et al. [16], this phenomenon is known to occur at very high phase ratios, particularly at the highest phase ratio of the PreDictor^{TM} isotherm plate where the resin volume is only 2 μL. The calculated binding capacity is much lower than that obtained from the initial isotherm and BTC measurements. This deviation is present for all four operators, which suggests that the actual resin amounts given may not be accurate, as the variation between operator measurements is very low (Coefficient of Variation <2%). The apparent lower binding capacity is not too critical in high-throughput screening, which is the main use of these plates. This is because it is mostly comparative, and differences between resins are still evident, even if the actual capacity is lower than in column experiments. The lysozyme and SP Sepharose FF model system may not be fully representative, and the results may vary depending on the type of resin used in the plates and the preparation procedure. These results should not be generalized to all microplate assays.

Fig. 4 shows a comparison of all resin preparation methods, the Predictor plate values and the reference value obtained from BTCs. The slurry-based method is close to the reference but is affected by the unknown void fraction in the settled bed. If assumed void fraction ε is increased from 0.3 to 0.4, the binding capacity starts to approach the value obtained from the breakthrough curve. However, in practice there is no easy way to obtain information on the void fraction in the settled bed. This directly affects the measured binding capacity by volume-based slurry preparation. The q_{max} value obtained from the PreDictor^{TM} plate measurements is very low and even when fitting the data

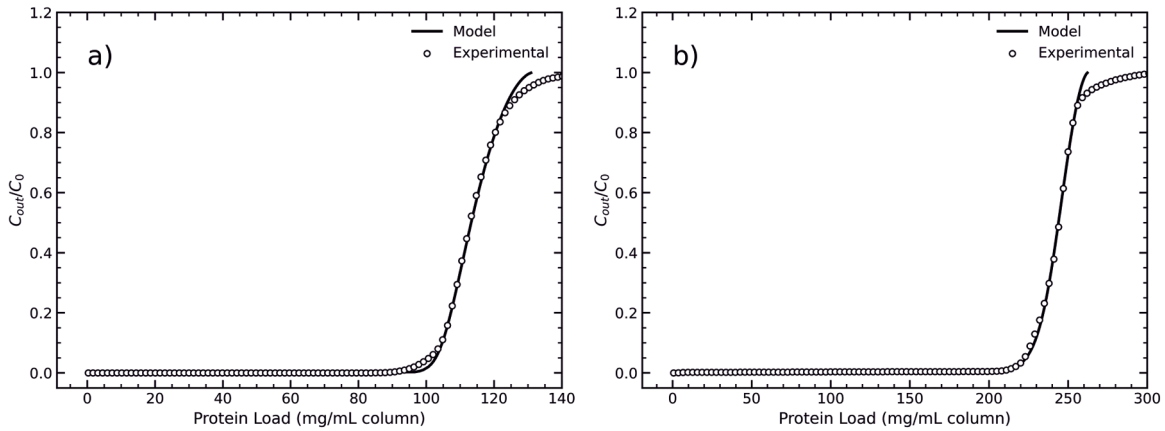

Fig. 1. Breakthrough curves for a) Lysozyme on SP Sepharose FF (CV= 2.5 mL) and b) GFP on Toyopearl Gigacap Q650-M (CV = 2.5 mL). The solid lines represent the constant pattern solution with pore diffusion developed by Weber and Chakravorti [13] fitted to the experimental data (*De_Lys*: 2·10-7 cm^2 / s, *De_GFP:* 4·10-7 cm^2 / s). The columns were operated at 4 minutes residence time.

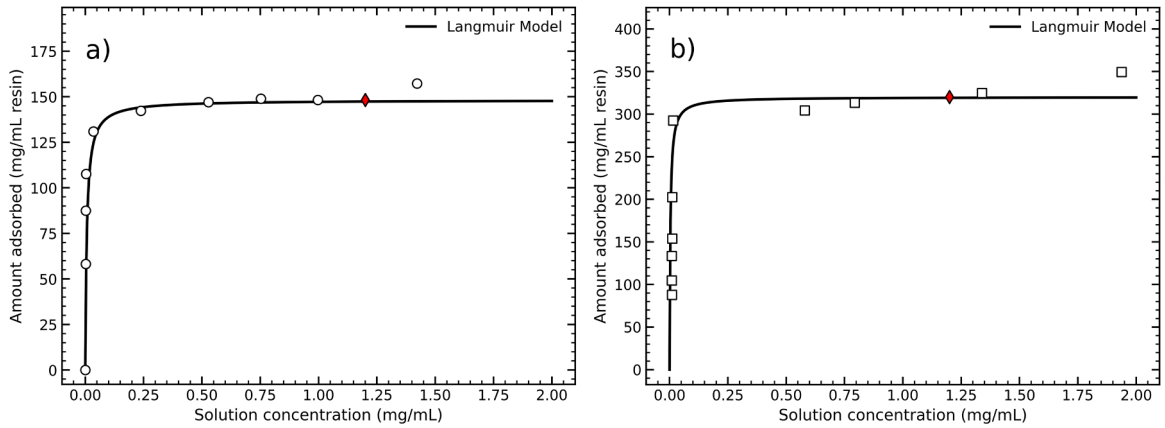

Fig. 2. Isotherms obtained by the slurry method of a) Lysozyme on SP Sepharose FF and b) GFP on Toyopearl Gigacap Q650-M. The red diamond marks the target phase ratio for the isotherm method comparison.

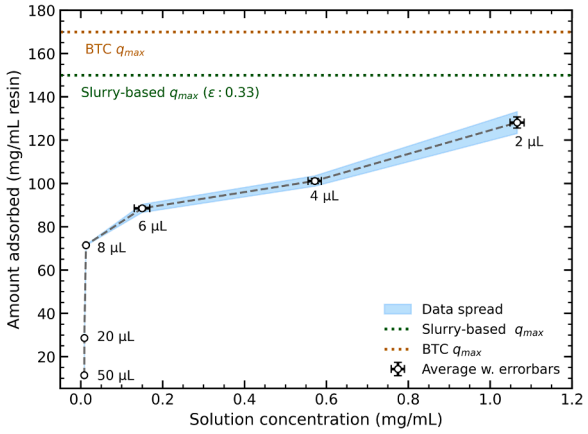

Fig. 3. PreDictorTM plate isotherm measurement, with data generated by four different operators. The shaded area represents the range of data for all operators. Resin volumes as reported by the manufacturer are shown for each data point. The target qmax of the breakthrough curve is shown in ochre and the maximum binding capacity obtained by the slurry-based method (assumed ε: 0.33) is shown in green.

average with the Langmuir isotherm produces only a q_{max} value of 125 mg/mL resin. If the 2 µL value is excluded the fitted value is even lower and close to 100 mg / mL resin.

Preparing resin by vacuum filtration encounters the issue of unequal drying of the resin. The resin on top of the vessel is much drier than the layers further down. This introduces a density gradient along the filtration device, making exact weighing of resin impossible. This is reflected by the trend seen in Fig. 4 showing values obtained by increasing drying time. Drying for 15 seconds obviously leaves too much liquid on the particles. This results in weighing in less resin by weight, reducing the measured binding capacity significantly. An upwards trend can be observed when increasing the filtration time. However, it is difficult to control the exact time point where all extra-particle fluid has been filtered out or dried off. This resin preparation method is difficult to transfer to a standardized operating procedure.

The value obtained by the centrifugation method comes closest to the binding capacity determined by the BTC method. This resin preparation method leads to a reproducible cake of hydrated particles with no liquid remaining in the extra-particle space, but with intraparticle space still saturated with equilibration buffer. Subsequently, the specific density of the hydrated particle can be determined with a pycnometer and isotherm measurements can easily be obtained by weighing the resin. However, there is still roughly a 10% difference between the q_{max} obtained by the centrifugation method and BTC, which could be accounted for by the compression factor of the resin. Fig. 4a shows that when accounting for bed compression by a factor of 1.09 during packing as outlined in the method section, we could match the BTC measurement almost exactly. The compression factor is unique to each resin and it is not feasible to perform such compression experiments routinely. However, even without adjustment by compression factor, the centrifugation method comes closest to the value obtained from column experiments.

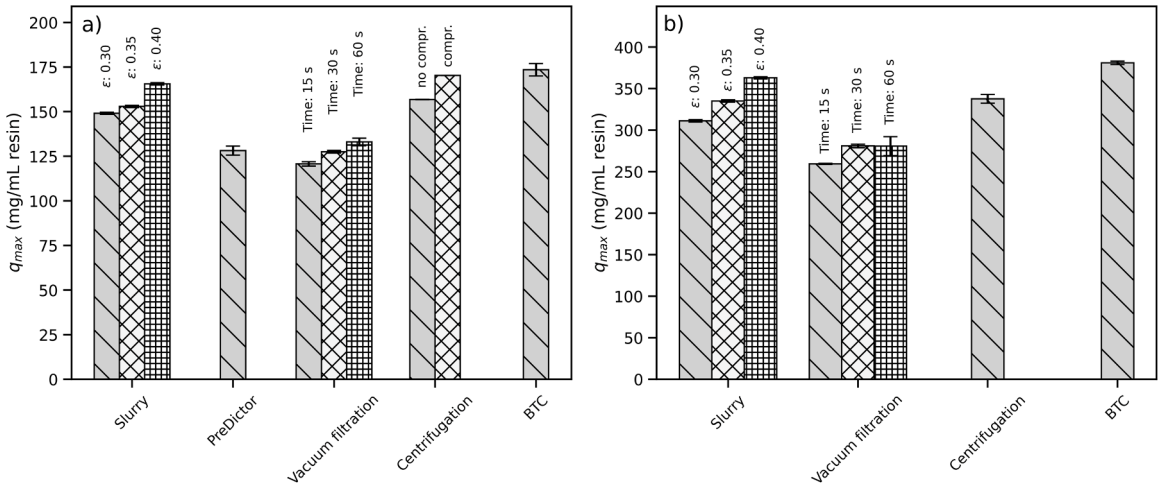

Fig. 4. Comparison of qmax determined by the methods compared to qmax of BTC for a) Lysozyme on SP Sepharose FF, b) GFP on Toyopearl Gigacap Q 650M.

Fig. 4b shows the comparison of binding capacity when applying the same resin preparation methods for studying adsorption of GFP on Gigacap Q650M. Toyopearl resins are based on methacrylate and are more rigid and thus more difficult to pack compared to agarose media [17,18]. However, the binding capacity trends of the isotherm methods are consistent to the one obtained from Lysozyme on SP Sepharose FF. Frequently, resin suppliers provide a factor for the additional amount of resin needed to pack a column to a specified bed volume. According to the packing instruction (TOYOPEARL and TSKgel Instruction Manual available from Tosoh Bioscience) this factor ranges from 1.1 to 1.25 depending on the type of Toyopearl resin. In our experiments, the difference of the obtained binding capacity between the BTC and centrifugation method was 1.13, which lies well within the given range of the manufacturer.

4. Conclusion

The results of this short study clearly show, that the method of resin preparation is crucial for determination of binding capacities in batch equilibrium binding experiments. Of all the methods tested, the combination of centrifugation, density measurement of the hydrated particles and weighing of the resulting hydrated particles best predicts the actual maximum binding capacity observed in column experiments. The volumetric slurry-based method also comes close to the expected q_{max} value, but is less precise, as the void fraction of settled bed used to prepare the slurry is not known with sufficient accuracy. It should be noted that our results, which ranked the centrifugation method as the most precise, were obtained using just two different types of resin. We underline that a larger dataset, incorporating a wider range of resins with various structural compositions, is required to confirm whether this trend is generally valid.

CRediT authorship contribution statement

Jürgen Beck: Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David Scheich:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Eric von Lieres:** Writing – review & editing, Conceptualization. **Rainer Hahn:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2025.466421.

Data availability

Data will be made available on request.

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