Foresight™ Chromatography Media Filter Plates

Instruction Manual

Catalog Numbers

732-4701	732-4712
732-4703	732-4714
732-4705	732-4716
732-4710	732-4718

BIO-RAD

Contents

Section 1
Introduction

The advent of high-throughput (HT) experimentation has revolutionized the conventional chromatographic process development paradigm. Traditionally, chromatographic process development data are obtained using column experiments alone.

These types of experiments usually consume significant amounts of sample, limiting the number of experiments that can be performed to define a given process design space.

Consequently, this design space is considered a nonoptimal space. HT experimentation substantially overcomes these limitations by allowing the execution of experiments in a miniaturized parallel format, yielding significant savings in time and material costs.[1-2]

To achieve this, HT experimentation utilizes microtiter plates and multichannel liquid dispensing tools (such as multichannel pipets and pipetting robotic platforms).

Two types of microtiter plates are commonly required, a filter plate containing the chromatographic media and a collection plate used to collect the sample. HT experimentation chromatographic experiments have been successfully used for isotherm determination,[3] method development and optimization,[4-5] and resin screening.[6] HT experimentation can be used with design of experiments (DoE), thereby becoming an essential tool to be employed in quality by design (QbD) and process analytical technology (PAT) approaches.[7-8]

This instruction manual describes how to use Foresight™ prepacked filter plates chromatography media.

Section 2
Theoretical Background

2.1 Batch Adsorption

Batch adsorption occurs when a liquid phase containing an adsorbate (i.e., target biomolecule, target impurity) comes in contact with a solid phase (i.e., chromatographic media) in a vessel under mixing. Batch adsorption is related to packed bed adsorption primarily in two ways.

First, the mechanisms for mass transfer and ligand adsorbate interaction are the same for both batch adsorption and packed bed adsorption, meaning the reaction is independent of the mode of operation. This means that the adsorption data obtained using plates can be used to describe the separation occurring in a packed bed column.

Second, a packed bed is an array of batch adsorption units connected in series with each unit representing a theoretical plate. In the case of a 96-well filter plate, a single well is a batch adsorption unit, hence a well is considered a theoretical plate.

2.2 Mass Balance

The mass balance of a solute between a solid and a liquid phase in a batch system is represented as follows:

$$\underbrace{V_0 C_0}_{\substack{\text{Amount}\\ \text{loaded}}} = \underbrace{V_{ads} q}_{\substack{\text{Amount}\\ \text{adsorbed}}} + \underbrace{VC}_{\substack{\text{Amount}\\ \text{unbound}}} \qquad \textbf{Eq. 1}$$

Where C_0 and C are the initial and unbound liquid phase concentrations, respectively. q represents the bound solid phase concentration, V_0 represents the initial liquid volume, and V represents the initial liquid volume minus the liquid volume in the pores of the media and the liquid held in the filter membrane.

Normally, V_0 is much larger than the latter two liquid volumes, hence the assumption that $V = V_0$ is a reasonable approximation. Implementing this assumption from Eq.1 leads to the following expression for the concentration of adsorbed solute (binding capacity):

$$q = \frac{V_0}{V_{ads}}(C_0 - C) = \frac{\text{amount bound}}{\text{volume of adsorbent}} \quad \text{Eq. 2}$$

According to Eq. 2, the amount of bound material can be calculated using the feed concentration (C_0), the flowthrough (unbound) concentration (C), the volume of sample added (V_0), and the volume of adsorbent present in a given well (V_{ads}).

A mass balance for multiple steps in a given well (e.g., flowthrough (FT) collection, washing, elution, and regeneration) is presented in Eq. 3a-3d:

$$m_{loaded} = m_{bound} + m_{unbound} \quad \text{Eq. 3a}$$

$$m_{loaded} = C_0 V_0 \quad \text{Eq. 3b}$$

$$m_{bound} = V_{wash} C_{wash} + V_{elution} C_{elution} + V_{regen} C_{regen} \quad \text{Eq. 3c}$$

$$m_{unbound} = V_{FT} C_{FT} \quad \text{Eq. 3d}$$

Where m is the mass in a given stage, C represents the solute liquid phase concentration in the various stages [i.e., loading (C_0), flowthrough collection (C_{FT}), washing (C_{wash}), elution ($C_{elution}$), and regeneration (C_{regen})], and V represents the liquid volume used in a given step [i.e., loading (V_0), washing (V_{wash}), elution ($V_{elution}$), and regeneration (V_{regen})]. The amount of bound protein can be estimated using either Eq. 3a or Eq. 3c; the amount of bound solute can be more easily estimated using Eq. 3a due to the simplicity of the experimental measurements required to obtain its inputs.

To use Eq. 3a, only the flowthrough concentration must be measured. Conversely, using Eq. 3c requires the solute concentration in the elution and regeneration steps be input. Thus, in order to use Eq. 3c, the experimental conditions should first be optimized to guarantee the total recovery of the solute.

Note: Calculating the amount of bound solute using flowthrough data (C_{FT}) and Eq. 3a is a faster and relatively simple approach.

Two process performance indicators can be calculated using the amount of bound solute (m_{bound}).

These indicators are recovery, Eq. 4a, and % yield Eq. 4b:

$$\text{Recovery} = \frac{m_{\text{bound}} + m_{\text{unbound}}}{m_{\text{loaded}}} 100\% \qquad \textbf{Eq. 4a}$$

$$\text{Yield} = \frac{m_{\text{eluted}}}{m_{\text{bound}}} 100\% \qquad \textbf{Eq. 4b}$$

2.3 Phase Ratio (β)

The phase ratio is defined as the ratio of the liquid to solid phase in a given well, represented as follows:

$$\beta = \frac{V_0}{V_{ads}} \qquad \textbf{Eq. 5}$$

Where β represents the phase ratio. The phase ratio is an important variable because: (1) it determines the slope of the operational line in a given isotherm; (2) it determines the level of depletion of the solute liquid phase concentration; and (3) it directly affects the magnitude of the experimental error incurred during HT chromatographic experiments.

For optimal results a phase ratio in the range of 20–40 is recommended.[9] To minimize the error, the final liquid phase concentration should be around 50% of the initial concentration, the exact value however depends on the precision of the protein concentration measurement[10]. Substituting Eq. 5 in Eq. 2 leads to the following expression:

$$q = \beta\left(C_0 - C\right) \qquad \text{Eq. 6}$$

This expression shows how the phase ratio and the adsorbed concentration are related. β directly affects the depletion level of the solute phase concentration, thus it has an effect on the uncertainty of chromatographic HT experimentation. Optimal values lie in the range of 20–40.

2.4 Adsorption Isotherm

An isotherm plots the solute solid and liquid phase concentrations at equilibrium for a given set of experimental conditions (e.g., pH, salt concentration).

The state of equilibrium, when the rates of desorption and adsorption are equal, may be reached between one and six hours of contact time in a Foresight™ prepacked filter plate (incubation time varies depending on target protein and buffer conditions. A 4–6 hr incubation time is recommended for conservative measure).

Figure 1 is a schematic representation of an adsorption isotherm. Figure 1A shows the operating line. This line connects the initial state (C_0, 0, t_0) with the equilibrium state (c_6, q_6, t_6). The slope of the operating line is equal to -β. During the adsorption process the adsorbed concentration increases along the operating line.

Figure 1A depicts six time points. The adsorbed concentration and the liquid phase concentration at equilibrium are represented by q_6 and c_6, respectively.

The time to reach equilibrium is represented as t_6.

Figure 1B shows three operating lines with distinct slopes. The slopes represent different values of β (phase ratio). Line I has the highest phase ratio, followed by line II and line III.

To work at the saturation capacity (q_{max}) use a small volume of media and a relatively high sample volume, which leads to a high phase ratio (Figure 1B, β_I). Working at the saturation capacity may be of interest when performing capacity (capture) studies.

Conversely, to operate under the saturation capacity a larger volume of media should be used, which results in a lower phase ratio. This is recommended when performing wash and elution studies (Figure 1B, β_I, β_{II}). This reasoning assumes that isotherm data for a given system are available.

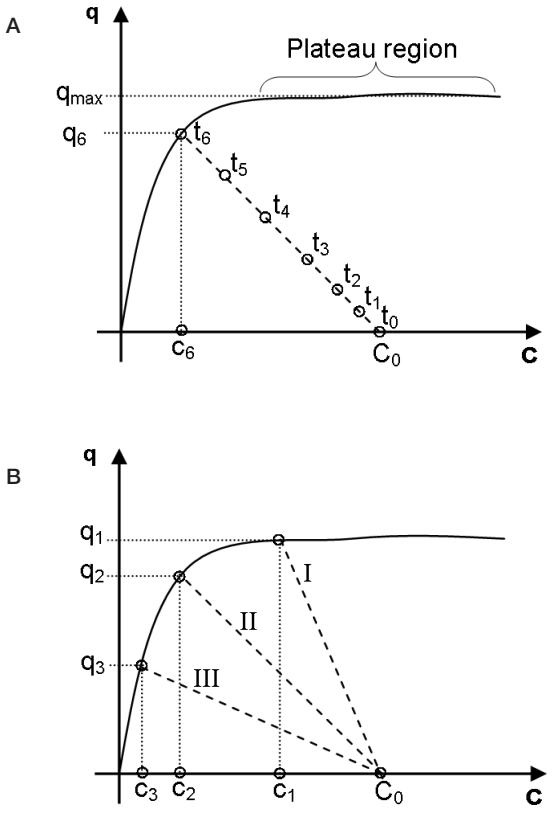


Fig. 1. Schematic representation of an adsorption isotherm. A, operating line describing the trajectory followed during the adsorption process, from the initial state to the equilibrium state. Dashed line: operating line; $t_{1...6}$: time points along the operating line; C_0: Initial concentration; q_1: solid phase concentration at equilibrium; c_1: liquid phase concentration in equilibrium with q_1; q_{max}: maximum binding capacity; **B,** operating lines with distinct slopes. Their different slopes are the result of using different phase ratios during the adsorption step. $\beta_I > \beta_{II} > \beta_{III}$.

Note:

1. The slope of the operating line is equal to the phase ratio (β).
2. The feed concentration and the phase ratio determine whether, after enough incubation time (i.e., 4–6 hours), media saturation will be attained (plateau of the isotherm).
3. A small volume of media relative to a fixed sample volume is recommended for capacity studies.
4. A larger volume of media relative to a fixed sample volume is recommended for wash and elution studies.

In sum, it is important to bear in mind that the capacity utilization for a given chromatographic medium is a function of the shape of the underlying solute isotherm and the magnitude of the phase ratio.

Several models are available to model an isotherm.[10–11] A model that is frequently used to represent adsorption isotherm data is the single component Langmuir isotherm Eq. 7.

$$q = q_m \frac{Kc}{1 + Kc} \qquad \text{Eq. 7}$$

Where q_m represents the maximum binding capacity, K represents the association constant; q and c represent the adsorbed and liquid phase concentrations at equilibrium. The two model parameters (q_m, K) can be estimated by parameter regression, for example in Excel using the solver add-in tool.

2.5 Uptake Curve

The uptake curve is constructed by taking time points along the operating line.

These points are created by loading the sample in different wells at predetermined time intervals.

The sample to be incubated the longest is added first.

The time interval used for sample addition dictates the number of time points. At the end of the longest incubation time the liquid in all wells is separated from the media, preferably through centrifugation.

The experiment uses a fixed phase ratio. The amount of bound solute is calculated using the mass balance presented in Eq. 2.

- A time between 20 and 60 minutes is usually enough for an uptake study. However, the actual value depends on the molecular properties, experimental conditions (e.g., ionic strength), and binding kinetics

- The contact time needed to achieve a target load can de determined using uptake curve data, for a given phase ratio and initial total solute concentration

2.6 Contact Time

The incubation or contact time refers to the time the sample containing the solute is in contact with the chromatographic media. Contact time affects the amount of solute bound to the media (capacity) for a fixed phase ratio and initial solute concentration. Relatively long incubation times lead to equilibrium capacities. Conversely, relatively shorter times lead to capacities located along the operating line Figure 1A.

2.7 Design of Experiments

Design of experiments (DoE) is a systematic investigation of the effect that inputs (experimental variables or factors) have on system outputs (responses). The main advantage of DoE is that a significant amount of information can be obtained in relatively few experiments. In sharp contrast with one-factor-at-a-time experimentation, in DoE all factors are varied simultaneously. DoE, generates process design spaces that support quality by design (QbD) and process analytical technology (PAT) approaches.

One of the most commonly used DoEs is the full factorial design. In this type of DoE, each experimental factor is considered at two levels (low and high) and the number of experiments is equal to 2^n, n being the number of experimental factors. Full factorial design is primarily useful when the number of factors is not very high. Other types of DoEs are available when the number of experimental factors is relatively high. DoE can be created and analyzed using commercially available statistical software packages. HT chromatographic experiments benefit from DoE in that one single media plate or part of it is sufficient for an entire DoE study.

2.8 Scaling up from Plates to Columns

HT chromatographic studies should be verified on prepacked columns to further optimize the design space. Foresight chromatography media columns prepacked with the same media used in the Foresight chromatography media plates are available in various scales. These columns are used for flow experiments, such as measuring the effect of of liquid residence time on dynamic binding capacity (DBC) at 10% breakthrough. Bio-Rad's prepacked columns are compatible with all liquid chromatography stations.

Section 3
High-Throughput Experiments

3.1 Manual Workflow

A manual workflow for HT experimentation starts with plate equilibration, followed by sample loading, incubation under mixing, and collection of unbound solute. These stages are common for uptake and isotherm experiments. For wash and elution experiments, the media plate (containing bound solute) is placed on top of a new collection plate. After the media plate is washed, the collected wash is analyzed. Next, the media plate is placed on top of a new collection plate for elution. The number of collection plates will be determined by the number of elution steps desired. Figure 2 shows the commonly encountered stages in a HT experimentation manual workflow. The schematic starts with sample loading, as it is assumed that the media plate has been previously equilibrated.

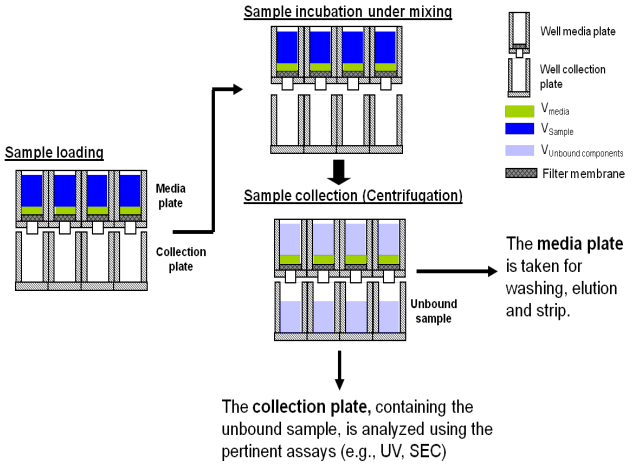


Fig. 2. Schematic representation of a HT experimentation manual workflow.

3.2 Types of Studies

3.2.1 Screening

To utilize the full potential of HT experimentation, statistical DoE is recommended. DoE identifies the experimental variables with the most significant impact on the experiment's output(s) in relatively few experiments. Examples of experimental variables include, but are not limited to, pH, conductivity/ionic strength, salt type, additives, and sample protein concentration.

To guarantee a desired load for the DoE work, a preliminary uptake kinetic experiment is recommended. This experiment provides the contact time required to achieve a given load and elucidates whether enough protein feed concentration was provided. If time and sample quantity allow, an additional isotherm experiment is recommended.

This experiment yields the range of feed concentration engulfing the plateau region of the isotherm, where the capacity is practically insensitive to changes in the feed concentration.

Note: A binding kinetic experiment (uptake curve) provides the incubation time required to achieve a desired load. Uptake data in juxtaposition with isotherm data provide a reliable way to determine the range of initial feed concentrations that leads to media saturation.

Screening studies can be used to find conditions that: (a) maximize load (binding of a target protein), (b) lead to flowthrough of the target or its similar impurities, and (c) lead to optimum levels of yield and purity. Furthermore, screening studies might also be used to scout for optimal cleaning conditions.

3.2.2 Adsorption Isotherm

An adsorption isotherm can be determined using a ladder of initial solute concentrations and a fixed phase ratio. This approach is suitable for plates loaded with a constant volume of media. Figure 3 presents plausible ways of configuring the Foresight™ chromatography media filter plate for an isotherm experiment done in triplicate. The presented examines the effect of salt concentration on the isotherm. The two alternatives depicted differ on whether an 8-(Figure 3A) or 12-(Figure 3B) channel pipet is available. To streamline the workflow, load the solutions in a 96-deep well plate, transferring them to the media plate using the multichannel pipet. The time required to reach equilibrium may range from 1–6 hours of incubation, depending on target protein and buffer conditions. The incubation should be done under mixing. To minimize evaporation, the media plate should be covered with a sealing film or a sealing mat.

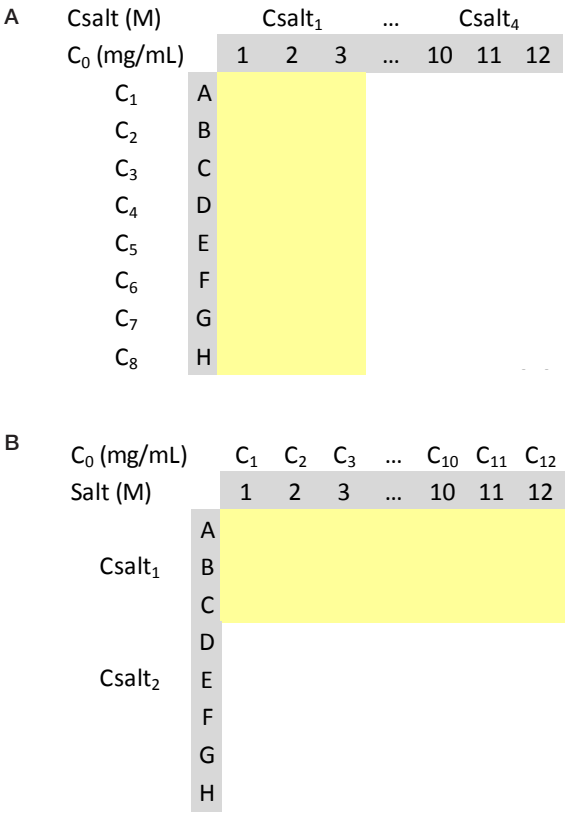
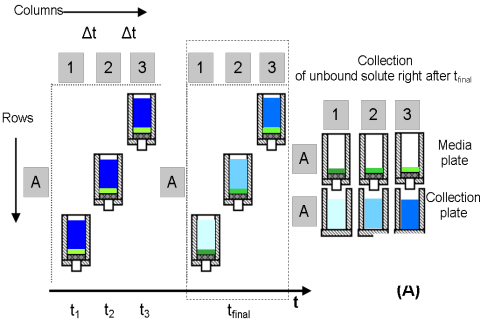


Fig. 3. Schematic representation of the plate configuration for isotherm experiments using a fixed phase ratio, and variable initial solute concentration (C_0). A, configuration suitable for an 8-channel pipet; **B,** configuration suitable for a 12-channel pipet. This example suggests performing three replicates for each salt concentration.

3.2.3 Binding Kinetics

This type of study is useful for defining the contact time of the batch adsorption experiment. For a fixed phase ratio and initial solute concentration, the contact time dictates how much solute will bind to the media. If there is enough sample and time, it is best to perform the binding kinetic study using two sufficiently distinct initial solute concentrations. This is especially useful if no isotherm data are available. The longest incubation time for uptake experiments is usually 60 minutes, meaning equilibrium is not reached, especially for macromolecules such as proteins and antibodies. Figure 4 shows the loading sequence (Figure 4A) and media plate configuration (Figure 4B) for the uptake experiments.

A



B

Loading Order		first						last
time (min)		t_1	t_2	t_3	...	t_{10}	t_{11}	t_{12}
C_0 (mg/mL)		1	2	3	...	10	11	12
	A							
C_1	B							
	C							
	D							
C_2	E							
	F							
	G	$\Delta t = t_{final}-t_{inital}/(N_t-1)$						
	H	N_t: Number of time points						

Fig. 4. HT uptake experiments. A, loading sequence of the plate. The color gradients indicate the level of saturation or depletion of the liquid and solid phases. The blue and green represent the liquid and media, respectively. In this example only three data points are shown, t_1 is the longest incubation time and t_3 the shortest; **B,** example of plate configuration for HT uptake experiments as a function of the initial solute concentration (C_0).

3.3 General Considerations

3.3.1 Sample

The sample should be clarified using a 0.45 or 0.22 µm filter prior to loading it to the media plate. Centrifugation prior to filtration is highly recommended for samples containing a relatively high concentration of suspended solids.

3.3.2 Media Plate Handling

The media plate should always be placed on top of a collection plate. Placing the media plate in direct contact with any surface can result in leakage of liquid from its wells.

To minimize evaporation during long incubation times, a self-adhesive microplate seal should be used to cover the media plate. This seal will also protect the media plate from contamination.

3.3.3 Liquid Transfer and Buffer Preparation

In cases where a pipetting robotic platform is not available, multichannel pipets should be used for liquid dispensing. Using 96-deep well plates to hold solutions that will be used in the media plate will save time.

3.3.4 Liquid Removal

The two alternatives for separating the liquid from the chromatographic media are vacuum filtration and centrifugation. Vacuum filtration is done using a 96-well plate vacuum manifold. Compared to centrifugation, vacuum filtration has a higher risk of well-to-well cross contamination during liquid removal due to the rheological properties of the solution to be vacuumed.

For instance, compared to a buffer solution, a protein solution of relatively high protein concentration will be prone to not forming clean single droplets on the tip of the drip director. The droplets tend to stick to the outer surface of the drip director, forming even bigger droplets, which may end up in multiple wells. In contrast, centrifugation does not have these shortcomings and is the preferred method for sample collection. Using a media plate with 0.8 ml well volume loaded with 0.3 ml of liquid and equipped with a 0.45 µm filtration membrane, a centrifugation cycle at 300 x g for 60–120 seconds should lead to complete sample collection. However this should be confirmed visually, since times up to 10 minutes are occasionally required. A swinging bucket rotor centrifuge, equipped with a microtiter plate bucket is recommended.

For steps such as plate equilibration, vacuum filtration can be used. Vacuum filtration for 1 minute (or until the wells are empty) at –1 to –3" in Hg is recommended. Visual inspection of the wells should be conducted prior to sample loading.

3.3.5 Mixing

An orbital shaker capable of mixing at 1100 rpm is recommended for good mixing. The shaker should be equipped with plate holders. The collection and media plate should be secured to each other and to the microtiter plate shaker before the start of the mixing cycle. The top of the media plate should be sealed.

3.4 Reliability of the HT experimentation Data: Error Analysis and Error Propagation

Errors are often associated with a mistake, but in science errors are not only the result of mistakes but also of the uncertainty associated with measuring. The best that can be done is to ensure that the error is small and to have a reliable estimate of it. This becomes even more relevant when the data reported is a calculated value, which depends on a measurable quantity and its corresponding uncertainty.

A suitable approach to estimate the error on a calculated amount is to sum the squares of the contributions of individual uncertainties, as shown in Eq. 8.[12]

$$f(x,y)$$

$$\sigma_{f(x,y)} = \sqrt{\left(\sigma_{f,x}\right)^2 + \left(\sigma_{f,y}\right)^2} \qquad \text{Eq. 8}$$

$$\sigma_{f,x} = \frac{\delta f(x,y)}{\delta x}\sigma_x \;\; ; \; \sigma_{f,y} = \frac{\delta f(x,y)}{\delta y}\sigma_y$$

Equations 9–11 were obtained by applying Eq. 8 to the calculation of the uncertainties (σ) on the corrected optical density (Eq. 9a–9b), the estimated protein concentration based on UV measurements, and the calculated bound protein concentration.

$$Abs_{\text{Corr}} = Abs_{\text{Sample}} - Abs_{\text{Blank}} \qquad \text{Eq. 9a}$$

$$\sigma_{Abs_{\text{Corr}}} = \sqrt{\left(\sigma_{\text{Sample}}\right)^2 + \left(-\sigma_{\text{Blank}}\right)^2} \qquad \text{Eq. 9b}$$

Where *Abs* represents the absorbance and σ represents its uncertainty.

$$C_P = \frac{OD_{\text{Corr}}}{m} \qquad \text{Eq. 10a}$$

$$\sigma_{C_P} = \sqrt{\left(\frac{1}{m}\sigma_{OD_{\text{Corr}}}\right)^2 + \left(-\frac{OD_{\text{Corr}}}{m^2}\sigma_m\right)^2} \qquad \text{Eq. 10b}$$

Where C_p and σ_p represent the calculated protein concentration and its corresponding uncertainty, respectively. *m* represents the slope of the calibration line (protein concentration vs. absorbance) and σ_m its corresponding uncertainty.

$$C_{\text{Load}}V_{\text{L}} = CV_{\text{L}} + qV_{\text{Media}} \qquad \text{Eq. 11a}$$

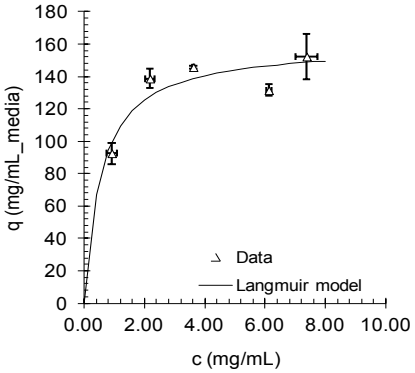
$$q = \frac{V_{\text{L}}}{V_{\text{Media}}}\left(C_{\text{Load}} - C\right) \qquad \text{Eq. 11b}$$

$$\sigma_q = \sqrt{\left(\frac{V_{\text{L}}}{V_{\text{Media}}}\sigma_{C_{\text{Load}}}\right)^2 + \left(-\frac{V_{\text{L}}}{V_{\text{Media}}}\sigma_C\right)^2} \qquad \text{Eq. 11c}$$

Where C_{Load} represents the protein load concentration, *C* the unbound protein concentration, and *q* the bound protein concentration. V_L and V_{Media} represent the volumes of liquid and media, respectively. All σ represent the uncertainty in each quantity.

In sum, using error analysis, the uncertainties of the measurable quantities are used to estimate the uncertainty of the calculated quantity. These expressions can be used, for example, to assess the experimental error of HT experimentation isotherm data in both coordinates, as shown in Figure 5.

A



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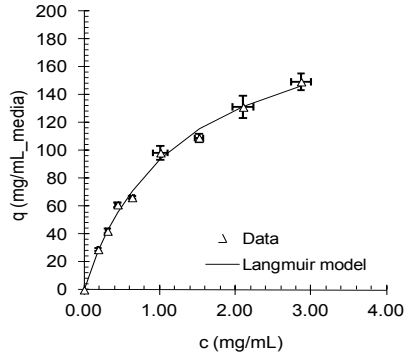

Fig. 5. Application of error analysis to HT experimentation isotherm data.
A, isotherm data on bovine serum albumin (BSA)-Nuvia Q-pH 8.5, q_{max} = 160 ± 12.3 mg/ml media; K = 1.80 ± 0.80 mg/ml; **B,** human immunoglobulin G (hIgG)-Nuvia-S-pH 5.0. q_{max} = 208 ± 11.7 mg/ml media; K = 0.82 ± 0.09 mg/ml.

3.5 Equipment and Materials Required for HT Experiments

In addition to the Foresight chromatography media plates the following equipment is required: (a) 96-well collection plates, (b) 8- or 12-channel pipets, (c) microplate shaker that can mix at 1100 rpm, (d) bucket centrifuge equipped with a rotor for microtiter plates, and (e) microtiter plate spectrophotometer.

HT experimentation analytics must accommodate and be optimized for a large number of samples of relatively small volume. Accessibility to an HPLC system equipped with a microtiter plate autosampler will increase the analytical throughput.

3.6 Detailed Experimental Protocol

3.6.1 Media Plate Preparation

a) Resuspend the medium to guarantee that media adhered to the plate's top seal returns to the well. Either one of the following two methods may be followed to ensure proper resuspension of the media:

- Gently tap filter plate on benchtop 20 times; or
- Centrifiguge plate at 100 x g for 60–120 seconds

b) Remove the media plate's bottom seal and place the media plate on top of its corresponding 96-well collection plate. Next, peel off the top seal while holding the media plate firmly.

c) Because the liquid storage solution does not contain valuable solutes, remove the solution by either vacuum filtration or centrifugation.

Recommended settings for vacuum filtration are 1 min at –1" Hg. If liquid is still visible in the wells of the media plate, repeat the cycle. Recommended settings for centrifugation are 1–2 min at 300 g (or more if visual inspection indicates residual liquid).

- The collection plate should be large enough to hold the storage solution. Remember to empty the collection plate when necessary

3.6.2 Plate Equilibration

Equilibrate the desired wells using 300 µl of buffer per well. Perform this procedure three times. During each equilibration, incubate the media plate on a microplate shaker for 1–2 min at 1100 rpm. Remove the equilibration buffer at the end of each equilibration step using either vacuum filtration or centrifugation.

- Firmly fix the media and collection plate to each other and to the microplate shaker

- Usually three equilibration cycles are enough to equilibrate the media. To monitor the progress, a micro pH probe can be used

Note: Remember to keep the media plate on top of a collection plate at all times.

After the last equilibration cycle, blot the media plate on a paper towel. Blotting eliminates droplets that might still be hanging on the drip directors, which will lead to non-reproducible dilution of the feed solution flowthrough. Blotting is recommended after the end of the sample incubation, washing, elution, and regeneration cycles.

3.6.3 Sample Loading

a) Volume and Concentration

- Load 300 µl of sample per well, cover the plate with a sealing film, and incubate it in the microplate shaker at 1100 rpm, for a predetermined incubation time

- The incubation time depends on the type of study and the target capacity utilization. For isotherm studies, one hr and up to six hr may be needed to reach equilibrium. For uptake kinetic studies 1–2 hr are recommended as the longest incubation time. For any other study an incubation time of 30–60 min usually suffices

- A volume of 300 µl translates to a phase ratio per well of 15 for the 20 µl media plates, respectively. These phase ratios generally lead to a decrease in the liquid phase concentration of less than 80%, which minimizes the experimental error

b) Collecting Unbound Material

- Remove the depleted sample using centrifugation. A cycle time of at least 2 min at 300 g usually removes all the liquid. Do not use vacuum filtration for this step. After collecting the liquid, label the collection plate “Flowthrough”, blot the media plate on a paper towel, and place it on top of a new collection plate

- The Flowthrough collection plate can now be analyzed, for instance for total protein concentration

3.6.4 Washing Weakly Bound Material

- Add 300 µl of wash buffer per well and cover the plate with sealing film. Incubate for 1 min at 1100 rpm. Next, transfer the media and collection plate to the centrifuge for liquid removal. Run a cycle at 300 g for typically 1–2 min. Do not use vacuum filtration for this step. After the liquid has been collected, label the collection plate “Wash”, blot the media plate, and place it on top of a new collection plate

- The Wash collection plate can now be analyzed. Additional washes can be performed; each should be collected in its own collection plate

3.6.5 Elution

- Add 300 µl of elution buffer per well and cover the plate with sealing film. Incubate for 1 min at 1100 rpm. Next, transfer the media and collection plate to the centrifuge for liquid removal. Run a cycle at 300 g for at least 2 min. Do not use vacuum filtration for this step. After the liquid has been collected, label the collection plate “Elution_1”, blot the media plate, and place it on top of a new collection plate (Elution_2)

- Usually three elution cycles are sufficient to recover the target molecule. Each elution cycle requires its own collection plate

Section 4
Application Example

4.1 IgG isotherm on cPrime™ Chromatographic Media

Introduction

Many different isotherm models have been proposed for the adsorption of proteins from a liquid solution onto a chromatography resin surface. The Langmuir model is widely used due to its ease of interpretation and its general agreement with experimental data. The Langmuir model is expressed by the following equation:

$$q = \frac{\mathrm{q_m KC}}{1 + KC}$$

Where q (mg/ml) and C (mg/ml) represent the adsorbed and liquid phase concentrations at equilibrium, respectively. q_m is the saturation capacity (mg/ml) and K the isotherm equilibrium constant (mg/ml), representing the affinity of the solute for the binding surface. q_m and K are the isotherm parameters, which can be estimated by fitting the Langmuir model to isotherm data.

This study used a Foresight™ chromatography media filter plate to determine isotherm data of a model molecule (human IgG) and a mixed-mode resin. The isotherm data were obtained using a ladder of initial hIgG concentrations and a fixed phase ratio. The isotherm data were fitted with the Langmuir isotherm model to determine the saturation capacity and the association constant.

Materials and Methods

1. Preparation of stock solutions:

   Loading buffer: 0.02 M Na Acetate, pH 4.5 was adjusted to a conductivity of 5.0 mS/cm with 0.02 M Na Acetate, 5 M NaCl, pH 4.5

   Stock protein solution: human IgG from Cohn fraction II+III (Sigma catalog #G4386-25G) was solubilized in the loading buffer to a final concentration of 3.0 mg/ml. The stock solution was thereafter sterile filtered.

2. Preparation of test solutions:

   Loading buffer was added proportionally into test tubes containing the stock protein solution to prepare 10 test solutions with a hIgG concentration ranging from 0.25 to 3.0 mg/ml. The tubes were vortexed prior to use. The total hIgG concentration was determined as follows: 200 µl of each of the test solutions was loaded to a 96-well plate to determine its absorbance at 280 nm. Next, the protein concentration was calculated using the Lambert-Beer law with a fixed mass extinction coefficient of 1.4 ml/mg/cm.

3. Generation of adsorption isotherm data using Foresight chromatography media filter plate:

   A 96-well plate containing Nuvia™ cPrime™, a mixed-mode resin was used. The sequence of steps was as follows:

   a) Apply 300 µl of loading buffer to each designated well. Apply vacuum to drain the loading buffer. Repeat twice.

   b) Apply 300 µl of test solution to each designated well. Secure the plate to microplate mixer and agitate at 1100 rpm for one hr. Longer incubation time may be required to reach equilibrium.

      A conservative incubation time of 4–6 hr is recommended for a broad range of buffer conditions and target molecules.

c) Collect the supernatant from each well by centrifugation (300 x g, 3 min) onto a 96-well collection plate.

d) Withdraw 200 µl of each supernatant and apply it onto a 96-well UV plate and measure the absorbance at 280 nm in each designated well. Calculate the protein concentration by using a mass extinction coefficient of 1.4. This concentration is equivalent to the equilibrium liquid phase concentration (C).

e) Calculate the protein adsorbed phase concentration using the following mass balance:

$$q = \frac{V_0}{V_{\text{ads}}}\left(C_0 - C\right)$$

Where *q* represents the absorbed phase concentration in equilibrium with *C*, C_0 represents the initial hIgG concentration, and *C* represents the liquid phase concentration at equilibrium.

f) Obtain the two parameters in the Langmuir equation by fitting the model to the isotherm data using the solver-add in Microsoft Excel. This process is done using as objective function the sum of squared errors.

Results and Discussion

The human IgG adsorption isotherm for mixed mode resin is shown in the figure. The corresponding fitted Langmuir adsorption capacity and the isotherm equilibrium constant are 50.7 mg/ml and 34.0 mg/ml respectively. The adsorption capacity calculated is consistent with a dynamic binding capacity of 43.1 to 53.8 mg/ml obtained in column chromatography.

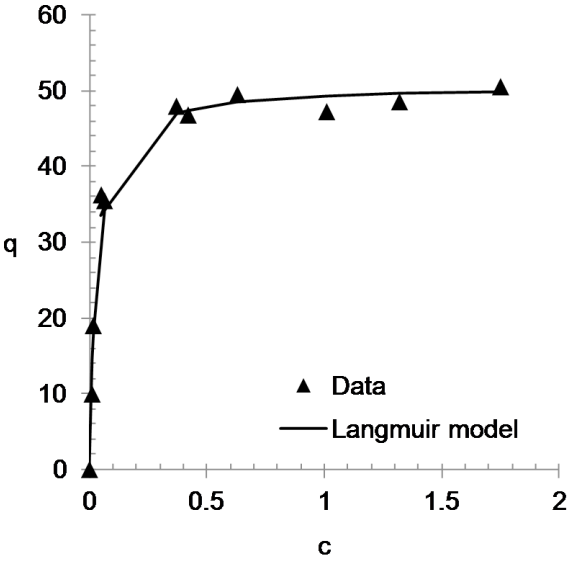

Fig. 6. HT experimentation Isotherm data for hIgG on Nuvia™ cPrime™ media, at pH of 4.5 and conductivity of 5.0 mS/cm. (▲) is isotherm data, solid black line is Langmuir model. q_{max} = 50.7 mg/ml ; K = 34.0 mg/ml.

Section 5
Ordering Information

5.1 Foresight™ Chromatography Media Filter Plates

Product	Mode	Catalog #
Foresight™ Nuvia™ S Filter Plate, 20 µl	Strong Cation	732-4701
Foresight Nuvia Q Filter Plate, 20 µl	Strong Anion	732-4703
Foresight™ Nuvia™ cPrime™ Filter Plate, 20 µl	Mixed Mode (Hydrophobic-Cationic)	732-4705
Foresight™ UNOsphere™ S Filter Plate, 20 µl	Strong Cation	732-4710
Foresight UNOsphere rS Filter Plate, 20 µl	Strong Cation	732-4712
Foresight UNOsphere Q Filter Plate, 20 µl	Strong Anion	732-4714
Foresight™ CHT™ Type I, 40 µm Filter Plate, 20 µl	Mixed Mode (Metal Affinity-Cationic)	732-4716
Foresight CHT Type II, 40 µmFilter Plate, 20 µl	Mixed Mode (Metal Affinity-Cationic)	732-4718

5.2 Foresight Chromatography Media Columns

Product	Mode	Catalog #
Foresight Nuvia S Column, 1 ml	Strong Cation	732-4720
Foresight Nuvia S Column, 5 ml	Strong Cation	732-4740
Foresight Nuvia Q Column, 1 ml	Strong Anion	732-4721
Foresight Nuvia Q Column, 5 ml	Strong Anion	732-4741
Foresight Nuvia cPrime Column, 1 ml	Mixed Mode (Hydrophobic-Cationic)	732-4722
Foresight Nuvia cPrime Column, 5 ml	Mixed Mode (Hydrophobic-Cationic)	732-4742
Foresight UNOsphere S Column, 1 ml	Strong Cation	732-4730
Foresight UNOsphere S Column, 5 ml	Strong Cation	732-4750
Foresight UNOsphere rS Column, 1 ml	Strong Cation	732-4731
Foresight UNOsphere rS Column, 5 ml	Strong Cation	732-4751
Foresight UNOsphere Q Column, 1 ml	Strong Anion	732-4732
Foresight UNOsphere Q Column, 5 ml	Strong Anion	732-4752
Foresight CHT Type I, 40 µm Column, 1 ml	Mixed Mode (Metal Affinity-Cationic)	732-4735
Foresight CHT Type I, 40 µm Column, 5 ml	Mixed Mode (Metal Affinity-Cationic)	732-4755
Foresight CHT Type II, 40 µm Column, 1 ml	Mixed Mode (Metal Affinity-Cationic)	732-4736
Foresight CHT Type II, 40 µm Column, 5 ml	Mixed Mode (Metal Affinity-Cationic)	732-4756

5.3 Foresight™ RoboColumns® Units

Product	Mode	Catalog #
Foresight™ Nuvia™ S, RoboColumn, 200 µl	Strong Cation	732-4801
Foresight Nuvia S RoboColumn, 600 µl	Strong Cation	732-4802
Foresight Nuvia Q RoboColumn, 200 µl	Strong Anion	732-4804
Foresight Nuvia Q RoboColumn, 600 µl	Strong Anion	732-4805
Foresight™ Nuvia™ cPrime™ RoboColumn, 200 µl	Mixed Mode (Hydrophobic-Cationic)	732-4807
Foresight Nuvia cPrime RoboColumn, 600 µl	Mixed Mode (Hydrophobic-Cationic)	732-4808
Foresight™ UNOsphere™ S RoboColumn, 200 µl	Strong Cation	732-4813
Foresight UNOsphere S RoboColumn, 600 µl	Strong Cation	732-4814
Foresight UNOsphere rS RoboColumn, 200 µl	Strong Cation	732-4816
Foresight UNOsphere rS RoboColumn, 600 µl	Strong Cation	732-4817
Foresight UNOsphere Q RoboColumn, 200 µl	Strong Anion	732-4819
Foresight UNOsphere Q RoboColumn, 600 µl	Strong Anion	732-4820
Foresight™ CHT™ Type I, 40 µm RoboColumn, 200 µl	Mixed Mode (Metal Affinity-Cationic)	732-4822
Foresight CHT Type I, 40 µm RoboColumn, 600 µl	Mixed Mode (Metal Affinity-Cationic)	732-4823
Foresight CHT Type I, 40 µm RoboColumn, 200 µl	Mixed Mode (Metal Affinity-Cationic)	732-4825
Foresight CHT Type I, 40 µm RoboColumn, 600 µl	Mixed Mode (Metal Affinity-Cationic)	732-4826

5.4 Related Products

Larger volumes for process scale are available. For more information on Bio-Rad's complete line of process chromatography media, visit us on the Web at www.bio-rad.com/process.

Section 6
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