Adsorption equilibrium isotherm studies using a high-throughput method

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This application note describes methods for determining adsorption isotherms using PreDictor™ 96-well filter plates and Assist software. PreDictor filter plates are designed for screening conditions for chromatography process development, while Assist software supports multivariate design of experiments and evaluation of collected data in terms of mass balance, adsorption isotherm estimation, and different types of plots.

In most cases of practical interest, chromatography column performance is dictated by the mass transfer and adsorption inside chromatography particles. These processes frequently occur at the same rate whether the chromatography medium is packed in a column or in a slurry in a microtiter plate well. Therefore, data necessary for process development can be collected from batch experiments performed in filter plates. A useful approach is to carry out initial screening in plate format, and once the screening is made, to continue process development using lab-scale chromatography, which gives more accurate data and also makes verification of the screening results possible. Numerous publications (e.g., 1, 2) have demonstrated that these miniaturized experiments generate useful data for design of column chromatography processes.

By carefully planning the experiments and systematically evaluating the data using suitable software, the overall result is comprehensive and covers a wide range of conditions that enables process understanding and the basis for further development.

An adsorption isotherm for a solute gives the equilibrium concentration of solute adsorbed at a given concentration in the liquid. The main purpose of studying isotherms is to identify conditions for which high adsorption capacities are obtained and to estimate the shape of the isotherm curve. The isotherms in this note consider a single target solute. In addition, the effect of other components present are included for the prevailing conditions. A more comprehensive isotherm study could involve varying the amounts of other components, but such a study would be beyond the scope of this application note.

The adsorption isotherm describes the phase equilibrium and can be used for process design

The isotherm is the result of the thermodynamics of the system, and as such it is a fundamental measure. At equilibrium, the adsorption and desorption rates balance each other and the result is shown as the isotherm. With knowledge of the isotherm, the effect of feed concentration on capacity can be estimated and can subsequently act as a guide in process design (e.g., allowing the choice of concentrating the feed before applying it to the chromatography column). Having isotherm data also gives an indication of what effect to expect, as well as an estimate of the robustness of the chromatographic step relative to variation in feed concentration.

The word ***isotherm*** is used for historical reasons; equilibrium of gas adsorbing on solids usually is described either at constant pressure and varying temperature (adsorption isobar) or at constant temperature and varying pressure (adsorption isotherm). In the case of adsorption from a liquid solution to a surface, concentration replaces the pressure. In such cases, pressure has little effect on the adsorption at a particular concentration. However, temperature may have a significant effect, so the experimental temperature should be recorded.

PreDictor

The migration of solute in a chromatography column is related to the isotherm shape. An isotherm can be favorable (concave), or unfavorable (linear or convex). A favorable isotherm can result in a sharp front of the migrating solute in a column (provided the mass transfer is not too slow), whereas an unfavorable isotherm always results in a dispersed front, and thus a shallow breakthrough curve. With knowledge of isotherm shape, the process designer can choose the best conditions for operating the chromatographic column.

For computer simulation of chromatography using a mechanistic model, the isotherm is needed, along with some data on the kinetics of the process. In Figure 1, simulated breakthrough curves for two different isotherms are shown. Only the isotherm parameters are different in the two cases, but the kinetics are the same. The effect on the breakthrough curve shape is dramatic, with the consequence that the dynamic capacity is 30%–40% higher for the case with the more favorable isotherm, although the static capacity is equal for the two cases at the feed concentration of 2.5.

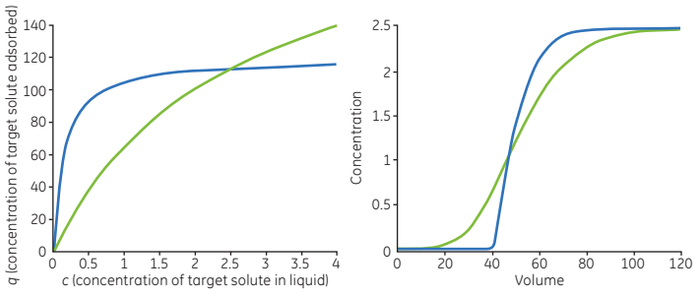

Fig 1. Two isotherms (left) and the corresponding calculated breakthrough curves (right). The more favorable isotherm (blue) results in a steeper breakthrough curve (blue). The 10% breakthrough level is reached at volume ~30 (green) and ~40 (blue) respectively.

Regulatory authorities increasingly demand knowledge about process fundamentals. The isotherm, carrying fundamental information about the process, is an important piece of such knowledge. Isotherms measured for a large design space can help in showing that the process has quality by design and that it is operated under optimal and robust conditions.

The isotherm can be determined in batch experiments by varying the solute mass or the phase ratio

Measuring the equilibrium concentration in a batch experiment is a straightforward method. It can be performed in a vessel with a known liquid volume and known amounts of solute and chromatography medium. Such batch experiments are simple to carry out using PreDictor plates – 96-well filter plates prefilled with chromatography media. PreDictor plates can enable substantial increases in efficiency by utilizing the following three techniques:

- **Miniaturization:** The small size requires little feed material – something that is important in early stages of drug development when feed supply is limited and many experiments have to be performed. Also, other chemicals are consumed in small quantities compared to regular column experiments.
- **Parallelization:** High-throughput batch experiments in plates can be carried out in parallel, for example by using manual or robotic multi-channel pipette and by using a UV plate reader. The liquid removal by centrifugation or vacuum is made in parallel.
- **Automation:** By using a robot, little manual handling is required, enabling high throughput of samples.

The isotherm experiment aims at finding values of the liquid concentration, *c*, and the corresponding concentration in the chromatography medium, *q*, at equilibrium.

Concentration *c* can be determined by analyzing the liquid supernatant after equilibrium is attained.

Concentration *q* can be indirectly measured by eluting the target molecule from the chromatography medium and determining the concentration in the eluate.

The concentrations are related by:

$$m_{added} = V_{added}c_{added} = V_{liquid}c + V_{medium}q$$

and thus, it is possible to calculate any of the variables if the others are known. In summary, the following alternatives can be used to determine the adsorption isotherm:

1) use the supernatant to find *c* and calculate *q* or

2) use the eluate to find *q* and calculate *c* or

3) measure both *c* and *q*

Alternative 1 can be recommended, since it requires less experimental work (only the flowthrough is needed) and possible errors introduced by subsequent experimental steps (e.g., washing the chromatography medium before elution) are avoided. Together, *c* and *q* constitute an isotherm data point.

The equilibrium concentration should be varied over a range of interest, for example liquid concentration from zero up to the maximal projected feed concentration for the chromatographic process. The variation can be achieved by varying any parameter in the above equation. Varying c_{added} is straightforward but requires preparation of several feed solutions – a process that is cumbersome and may introduce error. Varying V_{added} is simple, but only a small range is feasible because the slurry liquid volume has to be sufficient to allow for mixing and detection, and the volume in a well cannot be too high. Varying V_{medium} is also straightforward and is the recommended method. PreDictor isotherm plates are prefilled with a ladder of chromatography media volumes – 2, 4, 6, 8, 20, and 50 µl per well, for ease of use. This provides six isotherm data points to which a numerical model can be fitted, such as the Langmuir isotherm equation:

$$q = \frac{q_m c}{K + c}$$

where q_m is the maximum capacity and K is the dissociation constant. The Langmuir isotherm, when fitted to experimental data, thus yields a useful estimate of the maximal adsorption capacity.

Following liquid removal, there is always some liquid remaining in the filter and in the chromatography medium. This volume has to be considered when studying the equilibrium and mass transfer of the system. Measurements have led to the following expression (which is used in the Assist softare to calculate adjusted mass and adjusted capacity) for the liquid. The adjusted mass is the estimate of the mass not bound in the loading step.

$$V_{liquid} = V_{added} + 0.6V_{medium} + 6\ \mu l$$

Each combination of chromatography medium, feed composition, and conditions requires time to reach equilibrium. In practice, equilibrium is often reached well within six hours, but in some cases, more time may be needed. Longer times may be impractical, may reduce throughput and increase feed degradation, and may require time-consuming investigations of the kinetics. Liquid evaporation may introduce errors, although covering the microplates by foil (code number BR-1005-78) is recommended when evaporation may be an issue. In many cases, the absolute equilibrium does not have to be attained, but the analysis is a comparative screening, so shorter incubation times may be acceptable.

PreDictor Isotherm plates are prefilled with defined amounts of GE Healthcare chromatography media

Each well of a PreDictor plate has a filter in the bottom. The porous filter is such that the liquid in the plate can be removed by vacuum or by centrifugation, while the chromatography medium is retained in the wells, enabling further processing. The user can thus put the chromatography medium in contact with liquids in a series of stages, resembling the stages in a chromatography process.

Assist software – developed to help the chromatography process developer design and evaluate PreDictor plate experiments

When using Assist software, experimental design is intuitive and simple. Experimental data are loaded into the software for evaluation of mass balances, calculation of adsorption isotherms, and plotting of response curves and surfaces. A few examples of isotherm studies are outlined below. For further information about Assist software for evaluating adsorption isotherms, consult (4) or contact your GE Healthcare representative.

Adsorption isotherm of IgG on MabSelect SuRe

In order to study the equilibrium of human polyclonal IgG on the affinity chromatography medium MabSelect SuRe™, an experiment was set up as follows:

- The feed contained 3.9 mg/ml IgG in 20 mM PBS buffer (pH 7.4), 150 mM NaCl
- A PreDictor MabSelect SuRe isotherm (50/20/8/6/4/2 µl) plate (code number 28-9432-84) was used (i.e., the amount of MabSelect SuRe varied between wells)
- Each well was equilibrated with loading buffer (3 times 200 µl for 1 min each)
- Each well was loaded with 200 µl of the feed solution and incubated on a shaking table at 1100 rpm
- After incubation for 6 h, the liquid was removed by centrifugation and collected in a 96-well UV readable plate
- Each well was washed with loading buffer (3 times 200 µl for 1 min each) and all wash fractions were collected in UV readable plates
- Elution was performed with 3 times 200 µl of 20 mM citrate buffer at pH 3.6 and eluate fractions were collected in UV readable plates
- Concentration was determined from UV absorbance measured at 280 nm
- The isotherm was calculated from both flowthrough and elution data

The adsorption isotherm was determined at one common condition repeated for a total of 16 replicates. The concentration data from one plate collected during loading, (flowthrough), from three plates during wash, and from three plates during elution, were entered into the Assist software and analyzed in terms of adsorption isotherm. Langmuir isotherm parameter estimates are given in Table 1 and the resulting isotherm is shown in Figure 2. The flowthrough and elution data agree well; the small discrepancy observed is partly because the target molecule amount in the wash steps was not taken into account. The estimated maximum capacity is about 60 g IgG per liter chromatography medium and the isotherm is favorable.

Table 1. The estimated Langmuir parameter values for IgG on MabSelect SuRe

Parameter	Flowthrough	Elution
K (g/l)	0.1	0.2
q_m (g/l)	58	59

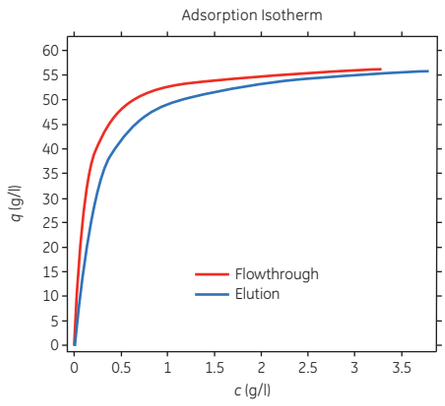

Fig 2. Isotherms for adsorption of IgG on MabSelect SuRe. The isotherms from the flowthrough and from elution data were determined using the Assist software.

Effect of NaCl concentration on equilibrium in PreDictor Capto Q isotherm plates

In order to study the effect of salt on the adsorption of BSA on Capto Q™, an experiment was set up as follows:

- The feed contained 3.6 mg/ml BSA in 50 mM acetate buffer (pH 4.25) and NaCl concentration up to 150 mM
- A PreDictor Capto Q isotherm (50/20/8/6/4/2 µl) plate (code number 28-9432-78) was used (i.e., the amount of Capto Q varied between wells)
- Each well was equilibrated with loading buffer (3 times 200 µl for 1 min each)
- Each well was loaded with 200 µl of the feed solutions and incubated on a shaking table at 1100 rpm
- After incubation for 3 h, the liquid was removed by centrifugation and collected in a 96-well UV readable plate
- Concentration was determined from UV absorbance measured at 280 nm

The experiments were performed at four different salt concentrations, with each condition performed in quadruplicate. The NaCl concentration levels investigated were 0, 20, 45 and 150 mM. The experimental data (flowthrough concentration, measured during loading) were entered in the Assist software and analyzed in terms of adsorption isotherm. The results show that increasing salt concentration results in decreased maximum capacity (Table 2 and Fig 3). For the case of no added NaCl, the isotherm is almost rectangular, (i.e., the ultimate favorable isotherm, with the estimated value of $K = 0$). For 20 to 45 mM, the isotherms are favorable, but for 150 mM, the data do not indicate a favorable isotherm. Instead, it is fairly linear (Fig 4, right side) and the maximum capacity q_m and dissociation constant K essentially lose their meaning.

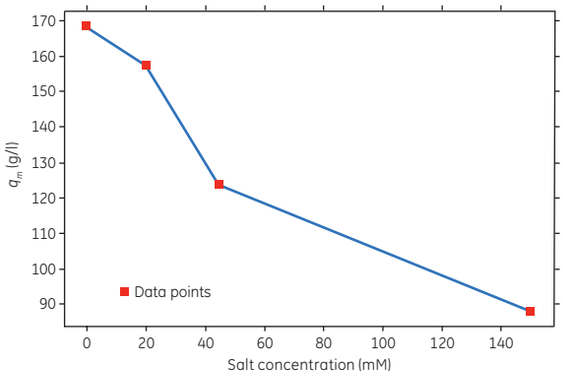

Fig 3. The Langmuir maximum capacity for adsorption of BSA on Capto Q for varying NaCl concentration. The isotherms from flowthrough data were determined using Assist software, and the fitted Langmuir isotherm gave the maximum capacity estimate.

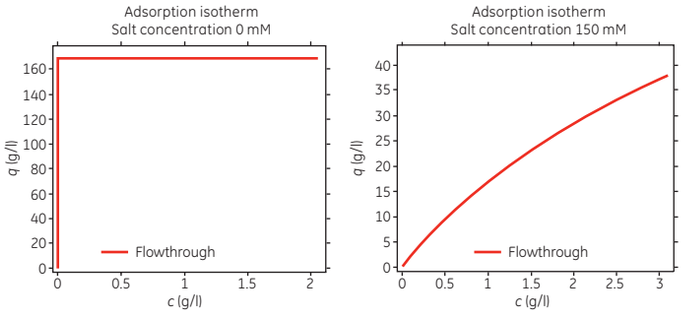

Fig 4. Elution data and fitted Langmuir isotherms for NaCl concentration of 0 mM (left) and 150 mM (right) showing a strongly favorable and an almost linear isotherm, respectively. The isotherms from flowthrough data were determined using Assist software.

Table 2. The estimated Langmuir parameter values for increasing salt concentration for adsorption of BSA on Capto Q

	NaCl concentration			
Parameter	**0 mM**	**20 mM**	**45 mM**	**150 mM**
K (g/l)	0	0.02	0.02	4
q_m (g/l)	168	157	124	89

PreDictor plates with constant chromatography medium volume can also be used for estimating isotherms

PreDictor isotherm plates, prefilled with chromatography media in volumes from 2 µl up to 50 µl, and Assist software were employed in the two studies above. The recommended procedure, that is fully supported by Assist software, is to use isotherm plates. It is also possible to use PreDictor plates with constant media volumes for determining adsorption isotherms. In order to compare the two methods, an experiment similar to the BSA example above was performed, but with PreDictor Capto Q (2 µl) plates (code number 28-9257-73). In order to fit the isotherm, the mass balance table was exported from Assist to a spreadsheet program and the Langmuir isotherm was fitted to the data. The results (Table 3) showed good agreement for q_m with the results from PreDictor isotherm plate data. Except for the case of highest salt concentration, q_m deviates less than 5% from the above values (Table 3 vs. Table 2).

Table 3. The estimated Langmuir parameter values for increasing salt concentration for adsorption of BSA on Capto Q using a PreDictor Capto Q (2 µl) plate

	NaCl concentration			
Parameter	**0 mM**	**20 mM**	**45 mM**	**150 mM**
K (g/l)	0.03	0.03	0.04	0.45
q_m (g/l)	169	154	117	46

Thus, the two methods are equivalent for determining q_m and it is a matter of preference when deciding which method to use, and also in what range the chromatography medium volume and the liquid volume need to be in order to investigate the relevant portions of the isotherm. In practice, PreDictor isotherm plates simplify the experimental work by taking the labor of feed dilution away, thus eliminating work and the potential introduction of error.

All *K* values for salt concentration up to 45 mM indicate a strongly favorable isotherm, whereas at 150 mM, the isotherm is essentially linear in the range studied.

Conclusions

Adsorption isotherms provide fundamental knowledge about the adsorption process and yield significant data for process design. With the increasing demands from authorities for process documentation around the process design space and with the general increasing demands for consistent quality, especially quality by design, each process requires more data in order to be approved. With the advent of robotic systems, microfilter plates, and efficient software tools, the process of developing chromatography processes has reached a new level of efficiency. Comprehensive information can now be gathered in a short time using little feed material. GE Healthcare supports the process development process with prefilled PreDictor plates and Assist software. Using PreDictor isotherm plates simplifies the workflow by making sample dilution series needless, thus reducing bench labor and eliminating a source of error.

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