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High throughput screening for optimization of purification process - CDMO perspective

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With the recent advances in biotechnology, there has been a considerable need for faster and more efficient development of biotherapeutics and their approval process. This translates to shorter process development timelines for both upstream & downstream operations.

As CMOs it becomes very essential to develop a platform approach involving a high throughput screening technology for evaluating multiple conditions for process optimization considering scalability along with manufacturability. With the development of small-scale multi-parallel bioreactor systems leading to screening of several high performing cell lines with high throughput technique, it becomes increasingly necessary to also have a similar throughput system for downstream purification process. Thus to cater to these needs high throughput process development has emerged as a predominant technique for downstream unit operations used by industry considering it to be cost efficient and also time saving methodology.

The high throughput process development technique for downstream unit operations are primarily used for chromatography stages which involves resin screening followed by optimization of process conditions (bind, wash and elution). At Kemwell we have optimized a high throughput screening (HTS) methodology which can be used for these purposes leading to significant decrease in process development timelines. The case study provided below focusses on optimization of high throughput method for resin screening.

CASE STUDY

Currently, increasingly high number of resins is available from different manufacturers and it is important to screen these resins for an optimum balance between binding efficiency and binding capacity to obtain maximum capacity utilization. This is more valuable for protein A resins since these are used for capture stage in the platform mAb process train and also has high impact on the manufacturing cost.

In this case study thermodynamic and kinetic effects of protein adsorption phenomenon for different protein A resins are evaluated which are the indicators for binding efficiency and capacity respectively.

Six resins were screened for protein A chromatography stage using the HTS technique. Micro Bio-Spin columns from BioRad were used which were packed with 10µl of resin per column. 400µl of protein solution at concentration of 0.5 to 2.5 mg/ml was added at different incubation times (5 to 120 mins) and the flow through was analysed for protein concentration.

Kinetic effects were evaluated through kinetic uptake curves plotted between binding capacity and incubation times at different concentration of load (C_0). Based on the kinetic uptake curves, the equilibrium stage or saturation capacity is attained for most of the resins at 120 mins of incubation time. A representative kinetic uptake curve at load concentration of 2.5 mg/ml is shown in **(Figure 1)**.

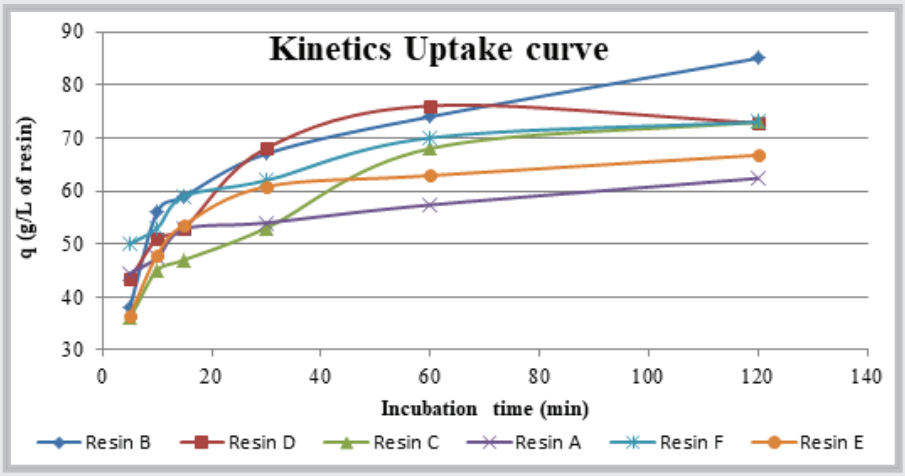

Figure 1 : Kinetics uptake curves at C_0 of 2.5 mg/ml

Thermodynamics of the protein adsorption phenomenon at equilibrium condition is evaluated by Langmuir adsorption curves **(Figure2)**.The adsorption isotherm curves are plotted between the binding capacity of the medium (q) and the protein concentration of flow through (C_{eq}) at equilibrium which was attained at 120 mins of incubation time based on kinetics uptake curve. The Langmuir isotherm is described by the equation given below

$$q = \frac{q_{max} C_{eq}}{K_d + C_{eq}}$$

q = Concentration of protein bound on the medium
q_{max} = Maximum saturation capacity
C_{eq} = Protein concentration at equilibrium
K_d = Equilibrium dissociation constant

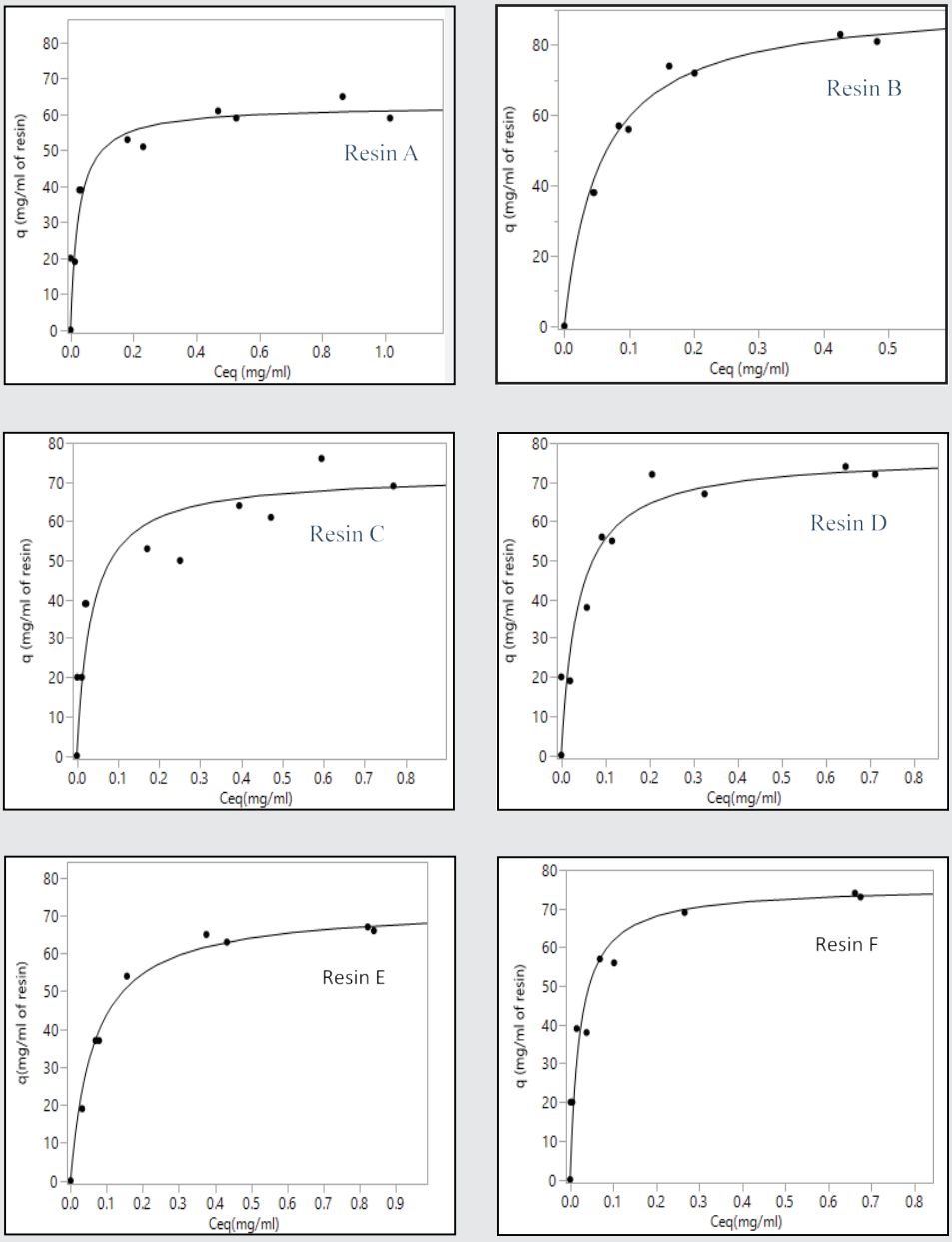

Figure 2: Adsorption isotherm curves

Adsorption isotherms having a large plateau phase and lower K_d value indicates good binding condition during which saturation capacity is achieved at low equilibrium concentrations of protein in flow through.

The K_d and q_{max} values for all the resins based on the adsorption isotherm curves are summarized below

Resin	K_d (mg/ml)	q_{max} (mg/ml of resin)
Resin A	0.03	65
Resin B	0.06	83
Resin C	0.04	76
Resin D	0.04	74
Resin E	0.07	67
Resin F	0.02	74

Resin A and F demonstrates good binding conditions with a larger plateau phase and lower K_d value. However Resin A also shows the lowest saturation capacity which indicates a lower binding capacity in dynamic condition. Resin B shows highest saturation capacity but comparatively lower K_d value which indicates binding capacity to be high but variable with residence time in dynamic condition.

Thus based on the above data Resin B and Resin F were chosen for further evaluation in dynamic condition by generating dynamic binding capacity (DBC) curves at column scale. Residence time of 4 minutes was used for the DBC study.

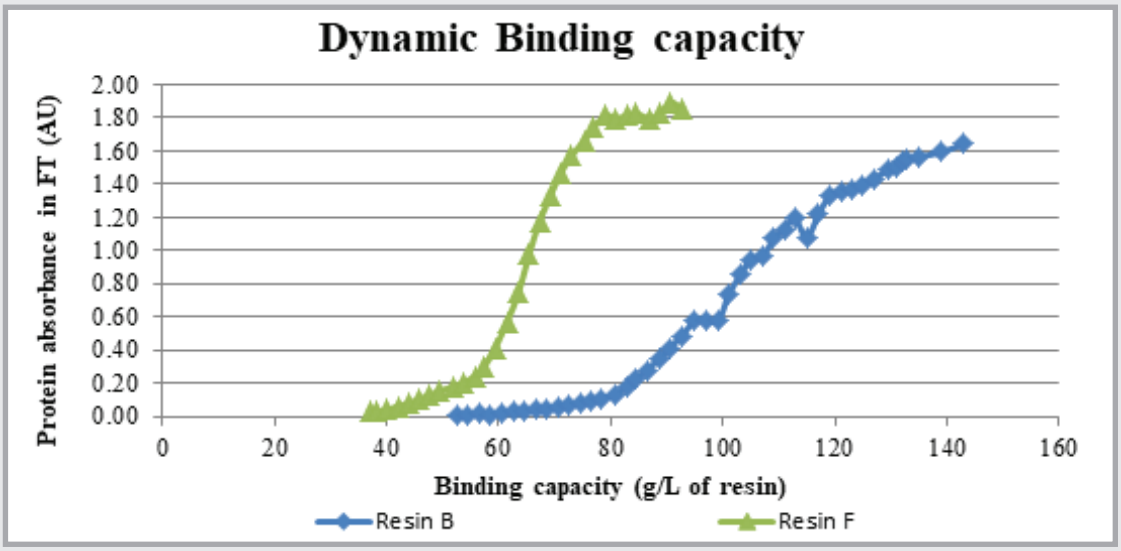

Figure3: Dynamic binding capacity curves of Resin B and Resin F

The DBC value obtained at 10% breakthrough for resin B and Resin F, are observed to be 84g/L of resin and 54g/L of resin respectively.

During the DBC study, saturation was observed to attain comparatively earlier for Resin F compared to Resin B during loading phase demonstrating comparatively better binding efficiency. However, Resin B shows significantly higher binding capacity. Thus observations incurred at column scale (dynamic condition) are correlating to the information obtained from the HTS study (static condition).

These resins can be further evaluated for wash and elution conditions to understand impact on yield and removal of process related impurities and accordingly the best resin can be selected for the process.

CONCLUSION

As observed from the above case study, high throughput methodology is a useful tool in screening different resins and can be employed for all chromatography stages. Moreover the outcome obtained from the high throughput screening studies can be also successfully extrapolated to higher scales or dynamic condition which is critical for further process finalization.

Apart from resin screening with respect to binding efficiency, the high throughput methodology can also be used for evaluating selectivity for impurity removal or control.

Considering the increasing number of resins available for all the chromatographic stages from different vendors, this tool provides the feasibility for obtaining information on thermodynamics of the protein–resin interaction and also selectivity with respect to impurity removal for multiple resins within a short period.

REFERENCES

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✓ **Jonathan L. Coffman, Jack F. Kramarczyk, Brian D. Kelley High-Throughput Screening of Chromatographic Separations: I. Method Development and Column Modeling; Biotechnology and Bioengineering, Vol. 100, No. 4, July 1, 2008**