**Impurity behavior in flow-through ion-exchange chromatography**

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Flow-through chromatography is naturally suited for high-productivity operations, but design challenges can hinder its implementation. Such challenges may be observed in monoclonal antibody (mAb) processing, where flow-through ion-exchange (IEX) is frequently used as a polishing step. An effective flow-through polishing step requires a large number of diverse impurities to bind the resin simultaneously, many of them often being dilute host cell proteins (HCPs) of unknown identity. Weak adsorption or product association may lead to the inadequate clearance of some HCPs, and this can be very sensitive to changes in the resin and solution conditions. Resin screening studies are normally performed during process development, but they do not provide transferable insights into HCP chromatographic behavior. Given the highly diverse nature of HCP profiles, the present study seeks to better understand the behavior of flow-through impurities in general. This could potentially help expedite process development, reduce costs, and increase consistency with quality by design principles. It could also help direct future column modeling efforts, which to date have almost exclusively focused on product molecules rather than impurities.

A general analysis is challenging for HCPs that persist via product association, which mechanistically varies with the therapeutic molecule, but it appears to be tractable for HCPs that persist via weak adsorption. Two attributes of the flow-through process make this the case. Flow-through IEX is an isocratic process, and impurities that persist via weak adsorption do so because they break through to exist beyond the column’s saturation front. They occupy a solution that is dilute with respect to adsorbing species, and their adsorption equilibria are therefore expected to be independent and linear. This study attempted to elucidate how transport and thermodynamic properties affect the breakthrough of weakly-adsorbing impurities, with the emphasis placed on contributions to the initial breakthrough volume, as the goal of flow-through polishing is the complete removal of trace impurities.

A picture containing graphical user interface

Description automatically generatedChromatographic breakthrough was first simulated using the general rate model and a Langmuir isotherm with parameters that are representative of previous calibrations to model protein systems. The feed concentration and adsorption equilibrium constant were varied, and breakthrough was observed to become quite diffuse for dilute species. This means that impurity breakthrough may occur at relatively low load volumes, even for moderately retained HCPs. To a good approximation, the initial breakthrough volume was shown to be described by:

|  |  |
| --- | --- |
|  | (1) |

Figure 1. Generalization of transport contributions to the breakthrough volume of dilute, weakly-adsorbing impurities. The slope term from eq. 1 is plotted on the ordinate, normalized by the ideal linear limit , where is total porosity. The particle Péclet number, , is plotted on the abscissa. Correlations are shown where is defined as 1% breakthrough (blue series) and 50% breakthrough (orange series).

Here, represents the initial breakthrough volume normalized by the column volume, is the column porosity, is the adsorption equilibrium constant, and is a slope term. This slope Chart, histogram

Description automatically generatedwas shown to be dictated by transport parameters, indicating that thermodynamic and transport contributions to the initial breakthrough volume are separable. Also, because transport contributions are described by a single factor, eq. 1 suggests that a dimensionless group may be able to generalize transport effects.

Correlation of simulation results showed transport contributions to the initial breakthrough volume were generalizable with the particle Péclet number (Figure 1). The generalization suggests a nonlinear increase in the initial breakthrough volume as the particle Péclet number is decreased. This provides a quantitative description of the inherent balance between separation performance and productivity. The effect of particle Péclet number was then validated using breakthrough experiments of lysozyme on SP Sepharose FF at the dilute load concentration of ~10 μg/ml (Figure 2), for which good quantitative agreement was obtained with corresponding column simulations. To more accurately model process conditions, the particle Péclet number effect was also validated with fluorescently-labelled lysozyme in the presence of a nonadsorbing mAb.

Figure 2. Experimental validation of the particle Péclet number effect. Breakthrough profiles are for lysozyme loaded on SP Sepharose FF at ~10 μg/ml at pH 7.0. Solid lines are experimental data, and dashed lines are from corresponding simulations. Simulation results for are not shown due to inaccuracies in modeling extra-column effects at low flow rates.

Chart, scatter chart

Description automatically generatedThe thermodynamic properties of weakly-adsorbing HCPs are often unknown, but correlations for HCP adsorption equilibria could be useful in estimating breakthrough volumes using eq. 1. As shown in the extant literature, adsorption equilibrium constants in IEX approximately follow a power-law in ionic strength, :

|  |  |
| --- | --- |
|  | (2) |

Here, the parameters and are related to adsorption strength and the protein “characteristic charge”, respectively. To explore possible correlations, equilibrium constants for model proteins on a few IEX resins were measured on-column with isocratic pulse retention times. The measured were regressed against ionic strength to extract power-law parameters, and a meta-correlation between the two parameters was discovered (Figure 3).

In principle, this correlation could be used to estimate adsorption equilibria as a function of ionic strength given one isocratic measurement of . However, a single measurement of elution ionic strength under a linear gradient would also suffice when coupled with an analysis using Yamamoto’s theory of linear gradient elution. Such an analysis was demonstrated using data from experiments and literature that show consistency with the identified meta-correlation. This suggests that a single linear gradient elution experiment followed by proteomic analysis could provide estimates for HCP adsorption equilibria as a function of ionic strength, as well as an estimate for linear gradient elution ionic strength as a function of gradient slope.

Figure 3. Meta-correlation of the parameters and , which are used to correlate IEX adsorption equilibrium constants as a function of ionic strength. Parameters were obtained by regressing experimental data for 16 protein-pH-resin systems.

To assess the meta-correlation’s general validity, retention factor data were compiled from experiments and literature containing over 200 protein-pH-resin combinations. Phase ratios specific to protein-resin pairs were unknown for the majority of these combinations, which precluded a true thermodynamic comparison based on equilibrium constants. Given this paucity, phase ratios were assumed to be of comparable magnitude, and retention factor data were directly regressed. The extracted power-law parameters were shown to follow the meta-correlation, albeit with more noise (Figure 4). Upon extrapolation, the correlation also agreed with parameters that were independently estimated from a continuum electrostatics model, suggesting that the meta-correlation is consistent with the physical basis for IEX adsorption.

Chart

Description automatically generatedUnfortunately, statistical uncertainty becomes problematic in practical application of the meta-correlation. Although the relationship between adsorption strength and characteristic charge is described qualitatively well, the quantitative description suffers from order-of-magnitude noise in the adsorption strength (parameter ), making the prediction interval of retention estimates too broad for practical utility. Additional factors are needed to explain some of the correlation’s noise. Analysis of covariance showed that the IEX type (cation/anion) significantly explains some noise (Figure 4), but the prediction intervals remain broad. This appreciable limitation precludes the correlation’s quantitative application to all HCPs generally. Nonetheless, it is helpful for understanding qualitative thermodynamic behavior, and it may be useful when subsets of protein-resin combinations are considered separately.

The complexity of adsorption phenomena, along with the diversity of HCPs encountered in flow-through IEX, currently makes a mechanistic model of HCP clearance elusive. However, this work has developed general insights into the chromatographic behavior of HCPs that persist via weak adsorption. The thermodynamic and transport contributions to the initial breakthrough volume of dilute impurities were found to be independent, and transport contributions were generalized with the particle Péclet number. The thermodynamic contribution was shown to be a linear dependence on the adsorption equilibrium constant, which is well known to follow a power-law in ionic strength. Although power-law parameters must be determined from experimental data, the meta-correlation identified here can eliminate one degree of freedom, thereby reducing the burden for approximately estimating adsorption equilibria to a single linear gradient elution experiment. Although approximate, these relationships provide novel insights into HCP chromatographic behavior, which may assist in the development of improved flow-through processes.

*Figure 4. Thermodynamic meta-correlation of power-law parameters describing the retention factor (k’) – ionic strength (IS) relationship of protein-pH-resin systems in the augmented data set. Correlations for cation-exchange (CEX) and anion-exchange (AEX) resins are shown as dashed lines. The dark shaded regions represent 95% confidence intervals, and the light shaded regions represent 95% prediction intervals. Note the abscissa is on a logarithmic scale.*

**Publication status**

A manuscript based on this work is in preparation. We expect to submit it to the Journal of Chromatography A by 9/30/21.