Mechanistic chromatography modeling - model selection

A mechanistic chromatography modeling workflow typically starts with the model selection step (Fig 1). This step includes selecting the column and pore model, accounting for the fluid dynamic effects in the chromatography column, as well as the adsorption isotherm which describes the interaction of biomolecule and ligand.

GoSilico™ Chromatography Modeling Software offers different mathematical equations to describe the fluid-dynamic and the thermodynamic effects inside the column.

As a rule of thumb, it is advisable to use a "bottom-up" approach starting with simpler models and increasing the complexity if the simulation describes the peak shape inaccurately. Complex models require more computational time and unreasonable parameter correlations, or unphysical parameter values might occur if certain parameters are unrealistic (e.g., dominant ligand shielding in low load experiments).

Column and pore models

Figure 2 shows a schematic representation of the effects occurring in a chromatography column. To select a suitable fluid-dynamic model (column and pore models), the influence of mass transfer limitation is important. There are various stages of complexity suitable for different resins and molecules.

The **Ideal Model** includes convection and adsorption to the stationary phase volume. As this model is simple, it is typically not applicable to preparative chromatography.

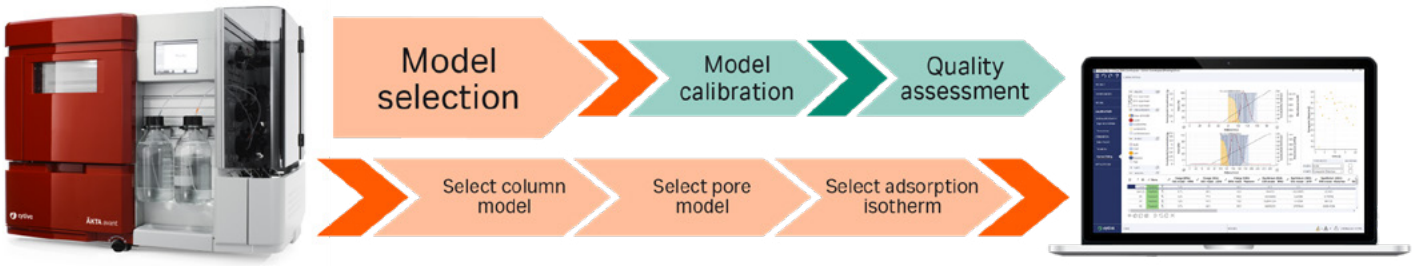

Fig 1. Outline of a mechanistic chromatography modeling workflow, with a focus on model selection.

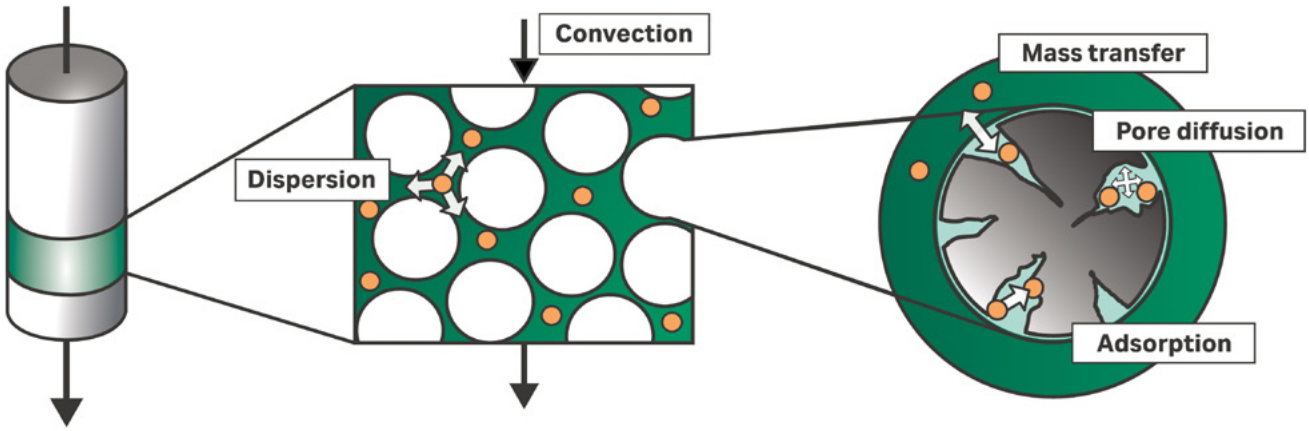

Fig 2. The basic principles within a chromatography column.

The **equilibrium dispersive model (EDM)** describes convection and axial dispersion. Film diffusion and pore diffusion are not included in the model, assuming there is no mass transfer limitation and thus it is sufficient to consider the total void volume and stationary phase volume (no differentiation between interstitial and pore phase). Consequently, the axial dispersion affects the total void volume in this model. Hence, the axial dispersion term used for EDM is the "apparent axial dispersion".

EDM is well suited to describe newer resins where no mass transfer limitation typically occurs due to the easy accessibility of the pores. This model is also applicable for modeling some smaller molecules such as insulin, as the mass transfer limitation is generally lower for molecules of smaller size. The EDM is not suitable if mass transfer and pore diffusion are rather slow, which leads to visible strong peak tailing in the chromatograms in linear gradient elution.

If there are mass transfer limitations, which is often observed for older resins and larger or more complex molecules, a more complex model to describe the fluid-dynamic effects is needed. In this case, the **Transport Dispersive Model Lumped Rate (TDM LR)** can be used. This model distinguishes between the interstitial and pore volume, and includes a separate parameter for the mass transfer between the two. As the pore diffusion is not considered separately, the film diffusion coefficient in TDM LR is a lumped parameter of film diffusion and pore diffusion and is the "effective film diffusion coefficient". The axial dispersion coefficient is then the "real axial dispersion coefficient" that is limited to the interstitial volume and can be determined from a larger tracer (e.g., dextran) experiment. TDM LR can be used to describe peaks with tailing that cannot be described by EDM.

If TDM LR is still not able to describe strong tailing or unusual peak shapes, the **Transport Dispersive Model General Rate (TDM GR)** may be used. Here, the film and pore diffusion are treated as two individual parameters. Because of the pore diffusion, an additional dimension in the radial direction of the beads is necessary. Therefore, the computational time is increased significantly. Consequently, TDM GR should only be used after TDM LR has failed. The predetermined parameters for TDM LR should be kept as constant as possible and only the mass transfer and pore-diffusion parameters should be refined.

Additionally, the GoSilico™ Chromatography Modeling Software comes with other models, which are applicable for special cases, such as models including the surface diffusion inside the pores or models using radial flow for some membrane capsules.

Isotherm models

To specify the thermodynamic effects during the adsorption to the stationary phase volume, GoSilico™ Chromatography Modeling Software offers also a variety of different isotherms for all kinds of interaction types. All isotherm equations can be found in the GoSilico™ Chromatography Modeling Software ***Help*** section, which is directly available in the software.

The most frequently chromatography mode is **IEX**. The **steric mass action model (SMA)** is a well understood isotherm from a mechanistic point of view. The SMA includes parameters to describe the binding kinetics, the equilibrium between adsorption and desorption, the characteristic protein charge, and a parameter to consider the ligand shielding due to steric hinderance and repulsion effects.

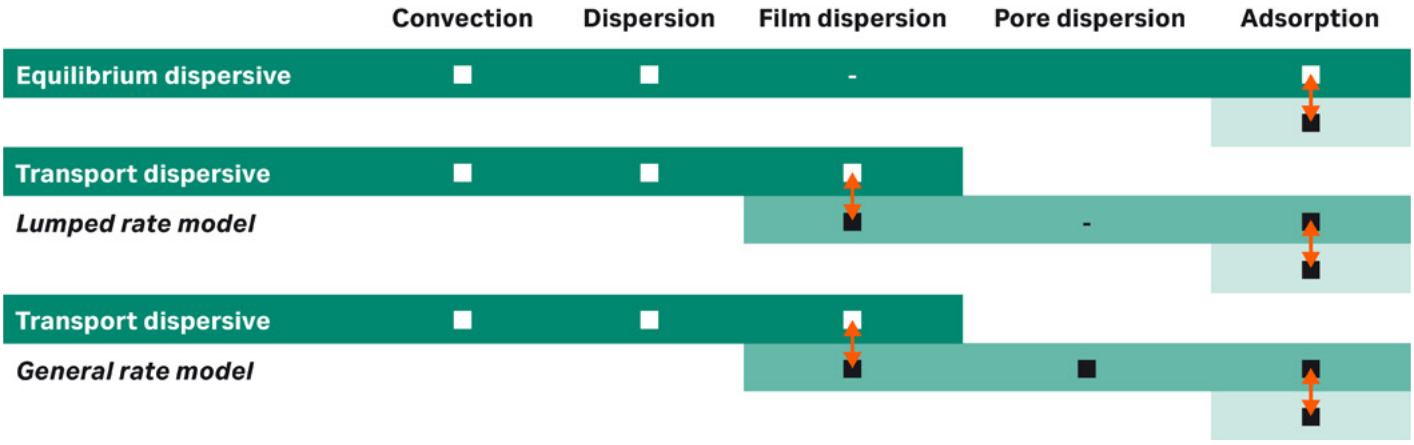

Fig 3. Overview of the effects to be considered for different column and pore models.

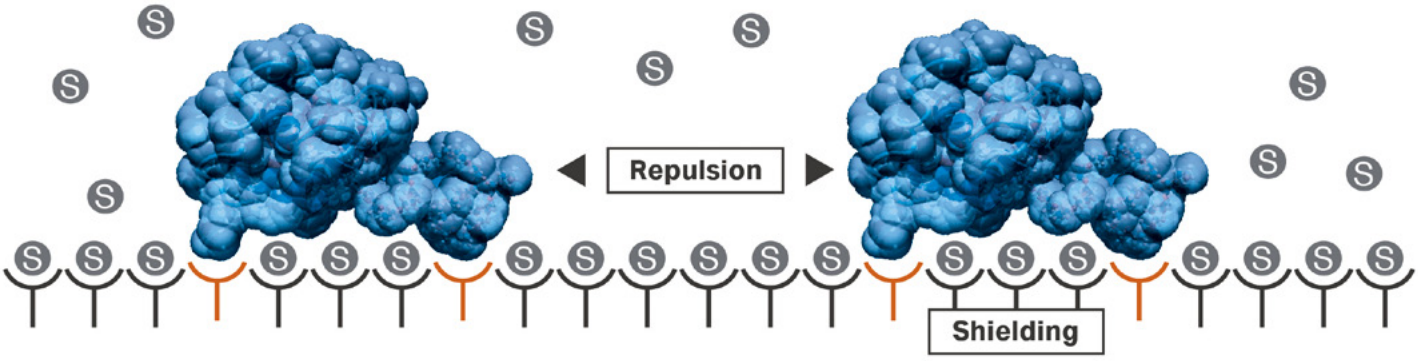

Fig 4. Illustration of steric hindrance effects of protein adsorption due to repulsion between proteins.

In some cases, the SMA is not sufficient, especially under high load conditions to capture all characteristics of asymmetric peaks due to the assumption of an activity coefficient equal to 1.0. To solve this, an SMA extension based on the generalized ion exchange isotherm is available (1). This includes an approximation of the activity coefficient including the influence of the salt and protein concentration. This isotherm might be better suited to describe trapezoidal or "shark fin" peak shapes. Both isotherms are also available including a linear pH extension. These isotherms may not be suitable for larger pH models.

Moreover, IEX can be modeled by a nonstoichiometric approach using the **Colloidal Particle Adsorption (CPA) isotherm** (2). Using the colloidal nature of proteins, the model allows a more fundamental description of interactions between proteins and charged ion adsorbers. Nonlinear adsorption effects are thereby ascribed to steric hindrance at the adsorber surface and electrostatic interactions between adsorbed proteins. In contrast to the SMA model, the maximum protein binding capacity of the adsorber is physically constrained by the adsorber surface area accessible to proteins, and not by its ionic capacity. This approach makes CPA suitable to describe unusual peak shapes occurring at high column loading or overloading of the column. Moreover, it is possible to describe larger pH ranges using the CPA isotherm.

Another chromatography mode often modeled is **HIC**. Essentially, there are two different isotherms to describe the protein-ligand interactions. The first one is the hydrophobic interaction isotherm, named HIC2008 (1), which is set up similarly to the generalized ion-exchange isotherm. The HIC isotherm introduces different fitting parameters to describe hydrophobic interaction between biomolecule and ligand.

The second isotherm developed for HIC, named HIC2016 (3) accounts also for the change in water structure during adsorption. Selecting, which isotherm is most suited to model the adsorption behavior depends on the project. If you have no previous experience, we suggest selecting the less complex isotherm (named "HIC2008" on GoSilico™ Chromatography Modeling Software) and switching to the "HIC2016" isotherm if modeling is not successful. Both isotherms are available with the linear pH extension to describe the influence of pH variations. As for the SMA isotherm and its extensions, the pH extension is most likely only valid for rather small pH ranges of +/- 0.5 pH unit.

In addition to IEX and HIC, the GoSilico™ Chromatography Modeling Software is used to **model multimodal (mixed mode) chromatography (MMC)**. In many cases, it is possible to describe MMC with an IEX or a HIC isotherm if one of the binding effects dominates. In the rare case of a change in the dominant effect from IEX to HIC, or *vice versa*, within the relevant process parameter range, there is an isotherm to take both effects into account. The mixed-mode isotherm, named MMC2010 (4), combines the SMA isotherm and the HIC isotherm (1) and results in a larger number of parameters. Therefore, the MMC2010 isotherm should only be used if both effects play a crucial role to avoid the risk of parameter overfitting.

Although possible, modeling of **affinity chromatography (AC)** is rarely employed since it provides little benefit compared with standard process development techniques. If modeling is used, the suitable type of isotherm is strongly dependent on the individual process. Langmuir-based isotherms can be used to describe Protein A chromatography. Also, HIC isotherms might work for certain applications.

In contrast to the other modes of interactions, modeling **reversed phase chromatography (RPC)** is especially challenging as gaps in the mechanistic understanding of effects taking place in RPC still exist. The influence of the detergents used in RPC is not yet well-understood. Therefore, the isotherm equations available for RPC are challenging to apply. Modeling RPC requires some experience as some workarounds might be needed. Hence, we do not recommend modeling RPC as a modeling beginner.

References

1. Mollerup, J.M. *et al.* Quality by design-thermodynamic modelling of chromatographic separation of proteins. *J. Chrom. A* 2008; 1177(2), 200-206. doi:10.1016/j.chroma.2007.08.059.

2. Briskot T. *et al.* Protein adsorption on ion exchange adsorbers: A comparison of a stoichiometric and non-stoichiometric modeling approach, *J. Chrom. A* 2021; 1653, 462397. doi.org/10.1016/j.chroma.2021.462397.

3. Wang G. *et al.* Water on hydrophobic surfaces: Mechanistic modeling of hydrophobic interaction chromatography. *J. Chrom. A* 2016; 1465, 71-78. doi: 10.1016/j.chroma.2016.07.085. Epub 2016 Jul 30. PMID: 27575919.

4. Nfor, B. K. *et al.* High-throughput isotherm determination and thermodynamic modeling of protein adsorption on mixed mode adsorbents. *J. Chrom. A* 2010; 1217(44), 6829-6850. doi:10.1016/*J. Chrom. A*.2010.07.069.

cytiva.com/modeling

CY25242-07MAR22-PD

cytiva