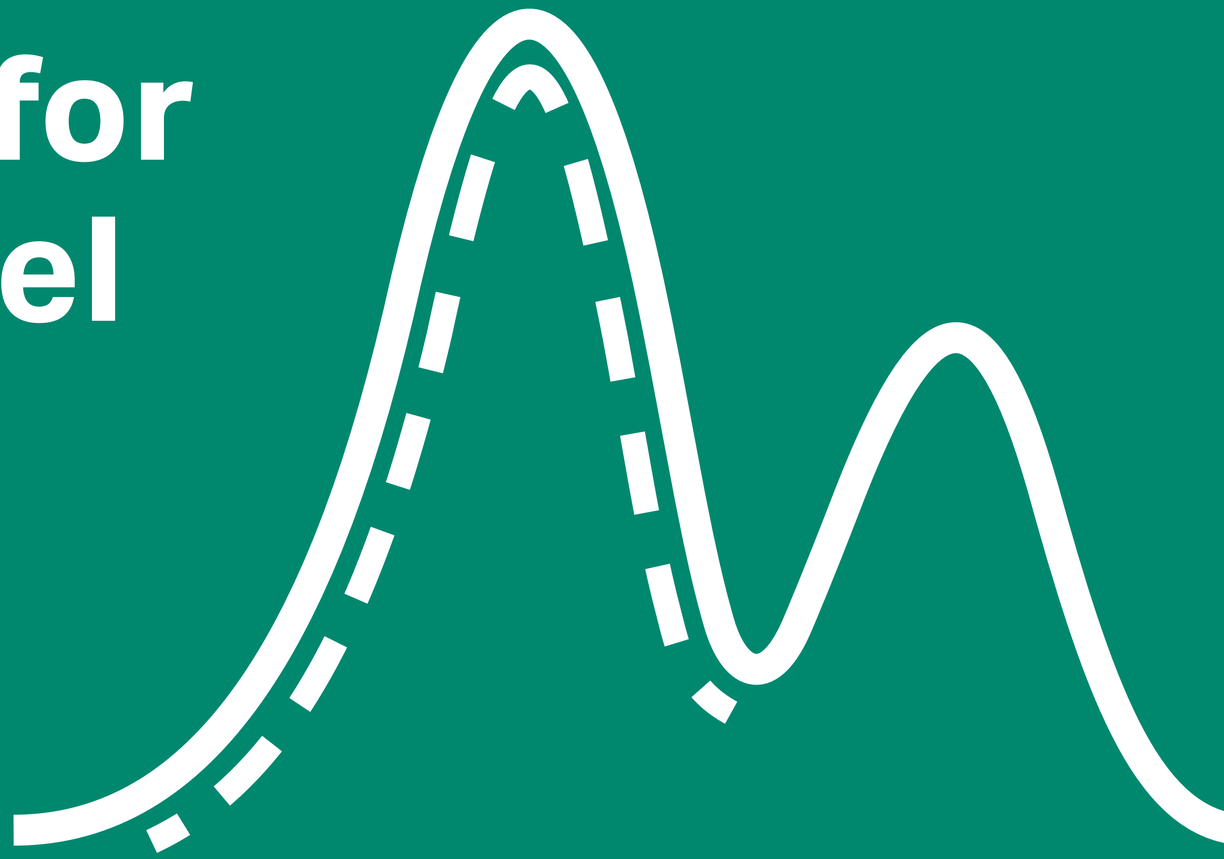
Principles and methods

System and column characterization for mechanistic model calibration



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Introduction

GoSilico™ Chromatography Modeling Software is a multi-purpose simulation software that provides tools for efficient and accurate solutions for a wide range of problems in liquid chromatography.

A model-based process development using GoSilico™ Chromatography Modeling Software starts with collecting experimental data to be used for model calibration. It is critical to carefully calibrate the system and column to ensure the model is an accurate representation of the physical process.

This document helps you to select or record the experimental data that are required or recommended for model calibration. We state whether a specific experiment or piece of information is mandatory, recommended, or optional for model building. This document serves as a general guide, and each model may require additional experiments not described herein to sufficiently calibrate it.

For a detailed overview of the implemented chromatography models and the theoretical background, see the software help document, which is accessible within the software.

01

System characterization

This section describes how to determine the relevant dead volumes of the chromatography skid using an ÄKTA™ system. This is important because dead volumes may cause some offsets in the chromatograms and may influence the calculation of column parameters (e.g., porosity) described in Section 2.

1.1 System dead volume (mandatory)

The system dead volume V_{sys} refers to the volume of the flow path from the point of sample injection to the detector (Fig 1). The point of sample injection is commonly the injection valve equipped with a sample loop and/or attached to a sample pump. The detectors are commonly the UV flow cell and the conductivity cell. For both these detectors, the dead volumes, $V_{sys,UV}$ and $V_{sys,cond}$, respectively, should be determined to ensure an accurate model. For the system dead volume characterization, the column is not used and should be replaced by a zero-volume connector, for example a 1/16-inch female to 1/16-inch female connector (product number 11000339).

The dead volume, $V_{sys,cond}$, is typically used to perform a sanity check of the entered salt events in the method. In a sanity check, simulated and measured conductivity traces are compared and, if an offset is visible, the volume axis can be corrected accordingly. $V_{sys,cond}$ and $V_{sys,UV}$ are also required for the porosity calculations in Section 2.4.

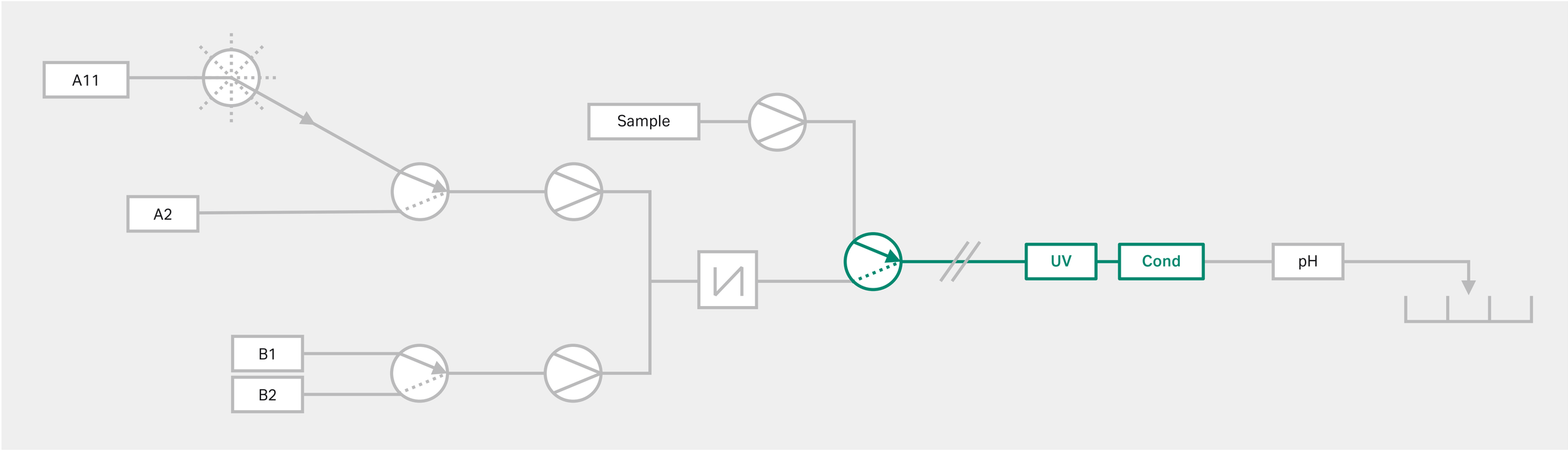


Fig 1. Schematic representation of the flow paths in an ÄKTA™ system. The path between injection valve and sensors is highlighted.

The system dead volumes are determined from the elution volumes of a tracer pulse injection. To ensure accurate model calibration, it is essential that the tracer does not change the viscosity of your fluid phase significantly and that it can be detected by the detectors attached to your chromatography skid.

Our recommendation is to use a 0.4 M NaCl background solution and a 1% acetone + 0.8 M NaCl solution for the injection. Acetone will be visible in the UV, while the NaCl pulse is only visible in the conductivity sensor.

Refer to Section 4 for example of experimental setup

Experimental setup

Running buffer	0.4 M NaCl
Tracer	0.8 M NaCl + 1% acetone

Experimental procedure

Phase	Variables
Method settings	Flow rate*: constant flow rate, the same as for small tracer experiment for column characterization Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: same position as for further experiments, without a column attached. The column inlet and outlet tubing is connected by a zero-volume connector
Equilibration	Equilibrate with at least 2× expected system volume
Sample application to fill loop†	Loop volume: for convenience, the same as for tracer experiments (in Section 2.2). Sample pump flow rate*: constant, same as system flowrate Fill loop with at least 10× the sample loop volume
Elution	Empty loop volume‡: at least 2× expected system volume to empty loop Elution volume: at least 2× expected system volume

* Volumetric flowrate presented is only valid for ÄKTA pure™ and ÄKTA avant™ systems
† Using a sample pump to fill the sample loop is preferred over manually filling the loop.
‡ Make sure that the sample application volume is greater than the expected system volume to avoid valve-switching mid-peak.

Experimental outcome

Extract the elution volumes from the injection to the peak maximum of the tracer peak.

$V_{sys,UV}$ = peak maximum of tracer recorded by the UV detector

$V_{sys,cond}$ = peak maximum of tracer recorded by the conductivity detector

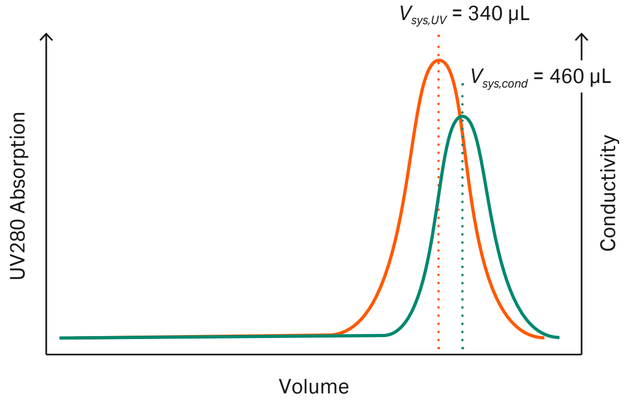
System dead volume characterization example

First, the system dead volumes from the injection valve to the conductivity and UV detector must be determined. This is done by a tracer pulse injection experiment. The conductivity sensor is typically slightly downstream of the UV detector. This experimental run is for a system calibration of an ÄKTA pure™ 25.

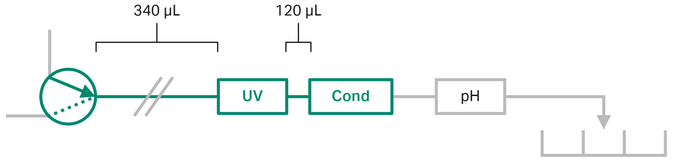
Eluent	0.4 M NaCl
Sample	0.8 M NaCl + 1% acetone (v/v)
Eluent	0.4 M NaCl

Phase	Variables
Method settings	Flow rate: 3 mL/min Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: 1
Equilibration	Equilibration volume: 1 mL
Sample application to fill loop	Loop volume: 200 µL Sample pump flow rate: 3 mL/min Fill loop with: ≥ 2 mL
Elution	Sample injection volume: 1 mL Elution volume: 1 mL

Tracer chromatograms evaluation



Parameter	Calculation
UV dead volume	Retention volume at peak max $V_{sys,UV}$
Conductivity dead volume	Retention volume at peak max $V_{sys,cond}$



Delay	Delay volume
From injection valve to UV detector	340 µL
From UV to Conductivity ($V_{sys,UV}$ – $V_{sys,cond}$)	120 µL

02

Column characterization

This section describes how to determine the essential column parameters such as porosities and fluid dynamic characteristics.

2.1 Column geometry and general adsorber properties (mandatory)

The column geometry and adsorber properties are required as input parameters for the GoSilico™ Chromatography Modeling Software to ensure accurate modeling of the fluid dynamic effects.

The column geometries are commonly obtained from your column's technical specification or supplier or can be measured (e.g., with a scale or a caliper).

The adsorber properties covered in this section are provided in the technical data sheets or specifications of your adsorber.

Geometries and adsorber properties

Column length/bed height, L	Distance between the upper and the lower piston of the column. To be measured; ideally with an accuracy of 1 mm or better.
Column volume	Cylinder volume calculated from L and D_i of the column $CV = \left(\frac{D_i}{2}\right)^2 \times \pi \times L$
Adsorber bead radius	The bead radius can be calculated from the mean particle diameter typically supplied in the technical sheets

Column geometry example

Column type	Capto™ S ImpAct, 15.7 mL*
Column dimensions	Tricorn™ 10/200, bed height 20 cm*
Bead diameter	50 µm
Column volume (calculated) CV	15.7 mL

*Relevant information can be found here: Tricorn™ Columns

Adsorber and column parameter example

Adsorber name	Capto™ S ImpAct
Adsorber size bead radius r_{bead}	$r_{bead} = D_{bead} \times \frac{1}{2}$ = 25 µm
Column name	Tricorn™ 10/200
Column bed height L	20 cm
Column inner diameter D_i	1 cm

More relevant information can be found at Capto™ S ImpAct

2.2 Small tracer injection (mandatory)

The small tracer retention volume V_{NaCl} refers to the total hold up volume of your chromatography column filled with the adsorber material. V_{NaCl} is used to determine the total porosity of the column. Consequently, the tracer must be of small molecular size so that the tracer penetrates the bead pores. To ensure accurate model calibration, the tracer should not adsorb onto the adsorber material, should not change the viscosity of the fluid phase significantly, but should be detectable by the detector that was used for system dead volume determination.

Our recommendation is to use a 0.4 M NaCl background solution and a 0.8 M NaCl solution for the tracer injection.

Experimental setup

Running buffer	0.4 M NaCl
Tracer	0.8 M NaCl

Experimental procedure

Phase	Variables
Method settings	Flow rate: constant (ideally process flow rate of biomolecule experiments) Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: column attached to the system, same position and tubings as for system characterization
Equilibration	Equilibrate with at least 2 CV of running buffer
Sample application to fill loop*	Loop volume: ~1%–2% of column volume Sample pump flow rate: constant, same as system flowrate Fill loop with: at least 10× loop volume
Elution	Sample injection volume: at least 3× loop volume Elution volume: 1.2 CV

* The use of a sample pump will allow for more consistent loop filling compared to manual injection.

Experimental outcome

Extract the elution volume from the injection to the peak maximum of the tracer peak $V_{NaCl.}$

2.3 Large tracer injection (recommended)

The large tracer retention volume $V_{Dextran}$ refers to the interstitial hold up volume between the adsorber beads. $V_{Dextran}$ is used to determine the interstitial porosity of the column. The tracer needs to have a sufficient molecular size so that it does not penetrate the bead pores. Based on the interstitial porosity and the total porosity, the bead porosity can be calculated. Like the small tracer, the large tracer should not adsorb or greatly change the viscosity of the mobile phase. It should be detectable by the detector that was used for system dead volume determination.

Our recommendation is to use a Dextran with a sufficiently large particle size solution, e.g., 10 g/L Dextran with a molecular weight of 2000 kDa. Please note that some labelled Dextran derivates such as Dextran blue may adsorb to your adsorber material (e.g., some anion exchange adsorbers). The recommended Dextran for the large tracer injection is commercially available from Sigma-Aldrich. If the Dextran peak intensity is too low for reliable peak readings, contact your Cytiva representative for assistance.

Experimental setup

Running buffer	0.4 M NaCl
Tracer	0.4 M NaCl + 10 g/L Dextran 2000 kDa

Experimental procedure

Phase	Variables
Method settings	Flow rate: Constant, 230 cm/h or maximum resin flowrate Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: column attached to the system, same position and tubings as for system characterization
Equilibration	Equilibrate with at least 2 CV of running buffer
Sample application to fill loop*	Loop volume: ~1%–2% of column volume, for convenience the same as in Section 2.2 Sample pump flow rate: constant, same as system flowrate Fill loop with: at least 10× loop volume
Elution	Sample injection volume: at least 3× loop volume Elution volume: 1.2 CV

* Using a sample pump to fill the sample loop is preferred over manually filling the loop.

Experimental outcome

Extract the elution volume from the injection to the peak maximum of the tracer peak $V_{Dextran}$

2.4 Column porosity determination

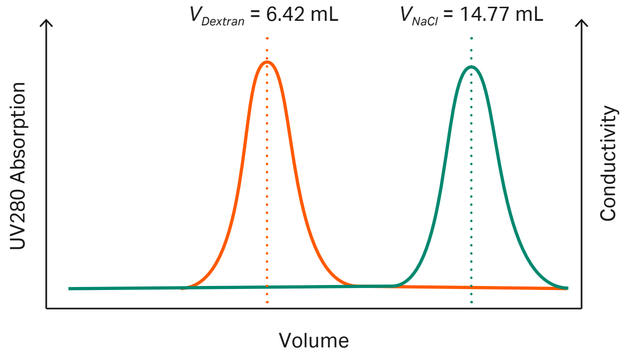
Total and interstitial porosity example

As with the system dead volume, the total and interstitial porosity is calculated from the elution volume at peak maximum of NaCl (small tracer) and Dextran (large tracer).

Eluent	0.4 M NaCl
Sample (*total porosity*)	0.8 M NaCl
Sample (*interstitial porosity*)	0.4 M NaCl + 10 g/L Dextran

Phase	Variables
Method settings	Column volume: 15.7 mL Flow rate: 3 mL/min Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: 1
Equilibration	Equilibration volume: 2 CV
Sample application to fill loop	Loop volume: 200 µL Sample pump flow rate: 3 mL/min Fill loop with: ≥ 2 mL
Elution	Sample injection volume: 1 mL Elution volume: 1.5 CV

Tracer chromatograms evaluation



Parameter	Calculation
Total porosity	$\varepsilon_{tot} = \frac{V_{NaCl} - V_{sys,cond}}{CV} = \frac{14.77 - 0.46}{15.7} = 0.91 =$ **91%**
Interstitial porosity	$\varepsilon_{int} = \frac{V_{Dextran} - V_{sys,UV}}{CV} = \frac{6.42 - 0.34}{15.7} = 0.39 =$ **39%**
Bead porosity	$\varepsilon_{bead} = \frac{\varepsilon_{tot} - \varepsilon_{int}}{1 - \varepsilon_{int}} = 0.85 =$ **85%**

Trouble shooting for large tracer injection

If Dextran is noisy, consider increasing the Dextran concentration or changing to a longer path length, e.g., from 2 mm UV path length to 10 mm.

2.5 Axial dispersion coefficient (recommended)

The axial dispersion coefficient D_{ax} can be derived from the broadening of the large tracer pulse (e.g., Dextran tracer experiment) as axial dispersion only occurs in the interstitial volume. So, the data collected in Section 2.3 can be reused for approximating D_{ax} by calculating the height of a theoretical plate (HETP) value. Typically, this is done by your chromatography system control software, for example, using the peak integration functionality of the UNICORN™ software from Cytiva. Reach out to the GoSilico™ software service team for any questions regarding the calculation of the axial dispersion coefficient.

Calculation procedure (preferred method)

1. Calculate HETP value of your large tracer pulse (e.g., in cm).
2. Calculate the linear flow rate (length/time unit, e.g., mm/s) from your volumetric flow rate (e.g., in mL/min) and your column cross sectional area (CSA): $u_{linear} = u_{volumetric}/CSA$.
3. Calculate the axial dispersion coefficient $D_{ax} = HETP \times 0.5 \times u_{interstitial}$ (e.g., in mm^2/s) where $u_{interstitial} = u_{linear}/\varepsilon_{interstitial}$.

Tracer chromatograms evaluation

The axial dispersion value of the column can be approximated from the HETP value of the interstitial porosity tracer peak.

The HETP value can be retrieved automatically form the peak integration tool in UNICORN™ software. In this example, it is 0.031.

Parameter	Calculation
Axial dispersion	$D_{ax} = \frac{u_{linear} \times HETP_{Dextran}}{2 \times \varepsilon_{int}} = \frac{230 \times 0.031}{2 \times 0.39} = 9.1\ cm^2/h =$ **0.252 mm²/s**

2.6 Ionic capacity (recommended)

The total ionic capacity, Λ, describes the number of ionic groups per adsorber backbone in IEX. This value is needed for several IEX isotherm.

You can derive the ionic capacity from the technical specifications of your adsorber or derive approximate values from literature or from previous projects with the same resin. You should keep in mind that the ionic capacity of a resin can differ significantly from lot to lot.

Please note that in almost all technical sheets, the ionic capacity (*IC*) is given per geometric column volume rather than adsorber backbone volume. You can convert these two definitions using the total porosity

$$IC_{backbone} = \frac{IC_{column}}{1 - \varepsilon_{tot}}$$

Sometimes, experimentally determined ionic capacity is not precise enough for calibrating a mechanistic model. However, literature data and technical specifications sometimes cannot describe the specific packed bed sufficiently. In these scenarios, the parameter can be refined (estimated) during the estimation process.

Experimental procedure

Consider whether you are working with a cation exchange chromatography (CEX) or an anion exchange chromatography (AEX) resin when performing the experiments. For an AEX resin, you need to equilibrate the column with NaOH and titrate with HCl.

Step	Eluent	Volume	Flowrate
Saturation	0.5 M HCl (CEX) 0.5 M NaOH (AEX)	5 CV	230 cm/h
Wash	Water	5 CV	230 cm/h
Titration*	0.1 M NaOH (CEX) 0.1 M HCl (AEX)	3 CV†	30 cm/h
Neutralization	0.5 M HCl (CEX) 0.5 M NaOH (AEX)	0.7 CV	230 cm/h
Wash	Water	5 CV	230 cm/h

* It is recommended to titrate from a Superloop™ assembly. 5 mL of titrant are injected in column bypass to prime the tubings.
† Can be adjusted depending on your resin

Experimental outcome

$$\Lambda_{backbone} = c_{NaOH} \times \frac{V_{NaOH} - V_{NaCl}}{CV \times (1 - \varepsilon_{tot})}$$

V_{NaCl} was experimentally determined; moreover, it can be calculated
$V_{NaCl} = \varepsilon_{tot} \times V_{column} + V_{sys,cond}$

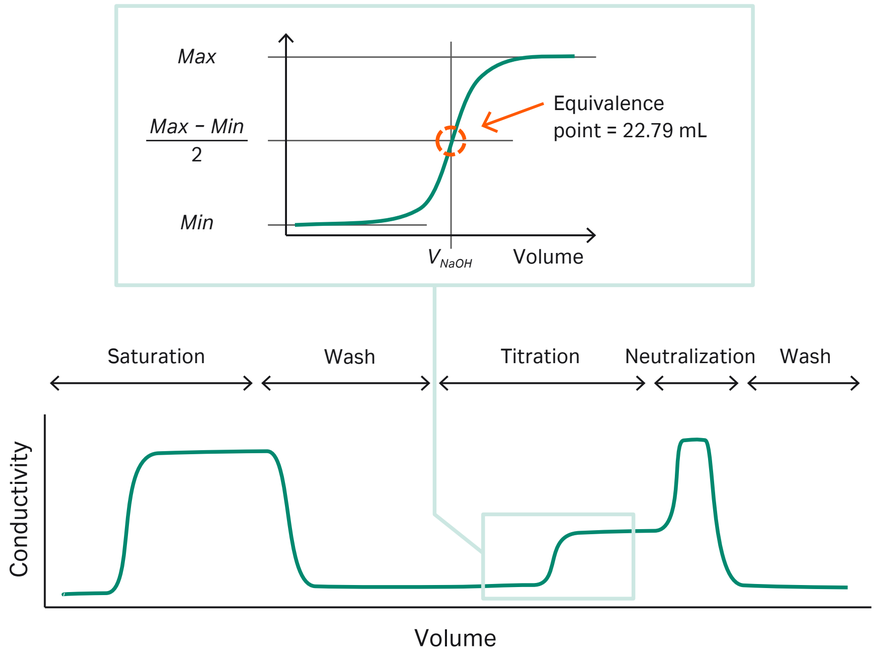
Titration example

The ionic capacity of the resin can be measured by on-column titration.

Phase	Eluent	Volume	Flowrate
Saturation	0.5 M HCl	5 CV	3 mL/min
Wash	Water	5 CV	3 mL/min
Titration	0.1 M NaOH	3 CV	0.4 mL/min
Neutralization	0.5 M HCl	0.7 CV	3 mL/min
Wash	Water	5 CV	3 mL/min

Titration experiment evaluation

The volume needed to titrate the resin is taken as the equivalence point (50% breakthrough) from the resulting titration chromatogram.



Parameter	Calculation
Ionic capacity	$\Lambda_{backbone} = c_{NaOH} \times \frac{V_{NaOH} - V_{NaCl}}{CV \times (1 - \varepsilon_{tot})} = 0.1 \times \frac{22.79 - 14.77}{15.7 \times (1 - 0.92)} =$ **0.64 mmol/mL**

2.7 Accessible surface area (optional)

The colloidal particle adsorption (CPA) model describes biomolecule adsorption in a non-stoichiometric way and introduces additional resin parameters other than porosity and ionic capacity, namely the accessible resin surface area, A_s, and ligand density, Γ_L. The apparent accessible surface area may be approximated from inverse size-exclusion chromatography as described in detail by DePhillips and Lenhoff (1).

The principle of inverse size-exclusion chromatography is to elute a set of differently sized probe molecules, often Dextrans, with narrow particle size distributions. Plotting the molecule size versus the fraction of accessible pore volume (K_D) yields the size selectivity curve of the resin. The size selectivity curve, along with a suitable model for simplifying the pore morphology, e.g., parallel cylindrical pores, may then be used to extract the apparent accessible surface area for a molecule of any size.

The ligand density of the resin is expressed as the number of charged ionic groups per surface area of the resin. This can be calculated by dividing the ionic capacity ($\Lambda_{backbone}$) by the total surface area ($A_{s,0}$) of the resin.

Deriving the accessible surface area experimentally is an optional experiment and is not mandatory for model building. Information about this parameter might also be found in technical reports or in literature. Especially in later cases, the A_s parameter can be refined (estimated) during the estimation process.

Experimental procedure

Eluent	0.4 M NaCl
Sample	0.4 M NaCl + 10 g/L Dextran SEC standards
Sample volume	~1%–2% of CV
Detector	Refractive index
Flowrate	30 cm/h
Equation	$K_{D,exp} = \frac{V_{Dextran\ SEC\ standard} - V_{Dextran}}{V_{NaCl} - V_{Dextran}}$

Calculation procedure

Step	Description	Equation
1	Calculate experimental pore distribution coefficient, $K_{D,exp}$, for all Dextran samples.	$K_{D,exp} = \frac{V_{Dextran\ SEC\ standard} - V_{Dextran}}{V_{NaCl} - V_{Dextran}}$
2	Estimate the hydrodynamic radius of all Dextran samples.	$R_h = 0.0271 MP^{0.498}$
3	Fit r_p and s_p to minimize *abs* ($K_{D,calc} - K_{D,exp}$) to get pore size distribution, *f(r)* R_h: molecule hydrodynamic radius *r*: pore radius	$K_{D,calc}(R) = \frac{\int_R^\infty (1 - \frac{R_h}{r})^2 f(r)dr}{\int_0^\infty f(r)dr}$ Where, $f(r) = \frac{1}{r\sqrt{2\pi}s_p} e^{\frac{(\ln(r) - r_p)^2}{2s_p^2}}$
4	Calculate accessible surface area per solid adsorber volume for a molecule with radius R_h.	$A_s(R_h) = \frac{\varepsilon_{tot} - \varepsilon_{int}}{1 - \varepsilon_{tot}} \frac{\int_R^\infty \frac{2(r - R_h)}{r^2} f(r)dr}{\int_0^\infty f(r)dr}$
5	Calculate total surface area, $A_{s,0}$ Or for example for a mAb, $A_{s,mAb}$	$= A_s(0)$ or $A_{s,mAb} = A_s(5.5)$
6	Calculate the ligand density from ionic capacity and total surface area.	$\Gamma_L = \frac{\Lambda_{backbone}}{A_{s,0}}$

1. DePhillips P, Lenhoff AM. Pore size distributions of cation-exchange adsorbents determined by inverse size-exclusion chromatography. *J. Chromatogr. A.* 2000;883(1-2):39-54. doi:10.1016/s0021-9673(00)00420-9

03

f(x) columns

This section gives a short overview about Cytiva *f*(x) columns.

3.1 Precharacterized *f*(x) columns

To ensure accurate model parameter values and reduce the work needed for column characterization, Cytiva also offers *f*(x) columns, which are prepacked and precharacterized lab scale columns for mechanistic modeling use. The columns are characterized with robust and standardized methods, allowing for reliable model building.

Cytiva *f*(x) columns are supplied with bed height; particle size; ionic capacity; surface area; ligand density; and total, interstitial, and bead porosity. These features allow a straightforward implementation into the mechanistic modeling workflow.

f(x) columns for mechanistic modeling of chromatography

04

Example: characterization at lab scale for cation-exchange chromatography

This section presents a general applicable example to determine the essential system and column parameters for a CEX column.

4.1 Example system and Capto™ S ImpAct column characterization

The following example shows the how to characterize a lab scale column packed with a strong cation exchange resin, including all mandatory and recommended experiments.

Resin	Capto™ S ImpAct
Column format	Tricorn™ 10/200 (15.7 mL CV)
System	ÄKTA pure™ 25

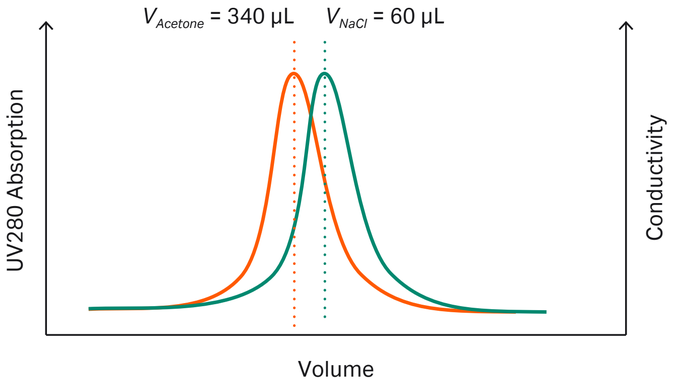
System characterization

First, the system dead volumes from the injection valve to the conductivity and UV detector must be determined. This is done by a tracer pulse injection experiment.

Use the resulting elution volume at peak maximum as the system dead volume.

Eluent	0.4 M NaCl
Sample	0.8 M NaCl + 1% acetone (v/v)

Phase	**Variables**
Method settings	Flow rate: 3 mL/min Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: 1
Equilibration	Equilibration volume: 1 mL
Sample application to fill loop	Loop volume: 200 µL Sample pump flow rate: 3 mL/min Fill loop with: 1 mL
Elution	Sample injection volume: 1 mL Elution volume: 1 mL



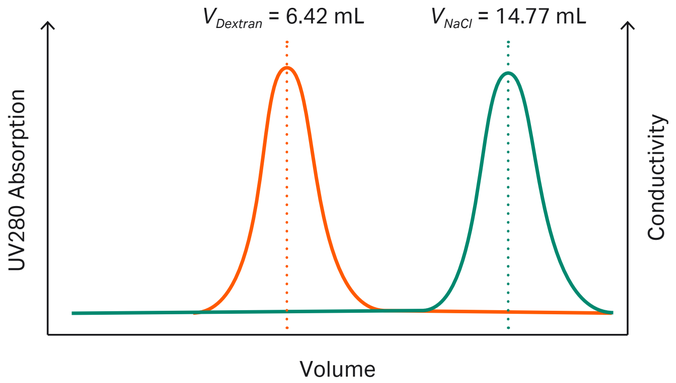
Total and interstitial porosity

After the system dead volumes has been determined, the porosities of the columns can be determined.

As with the system dead volume, the total and interstitial porosity is calculated from the elution volume at peak maximum of NaCl (small tracer) and Dextran (large tracer), respectively.

Eluent	0.4 M NaCl
Sample *(total porosity)*	0.8 M NaCl
Sample *(interstitial porosity)*	0.4 M NaCl + 10 g/L Dextran

Phase	**Variables**
Method settings	Flow rate: 3 mL/min Flow direction: Down flow pH electrode: Off-line Restrictor: In-line Column position: 1
Equilibration	Equilibration volume: 2 CV
Sample application to fill loop	Loop volume: 200 µL Sample pump flow rate: 3 mL/min Fill loop with: 1 mL
Elution	Sample injection volume: 1 mL Elution volume: 1.5 CV



Axial dispersion

The axial dispersion value of the column can be approximated from the HETP value of the interstitial porosity tracer peak.

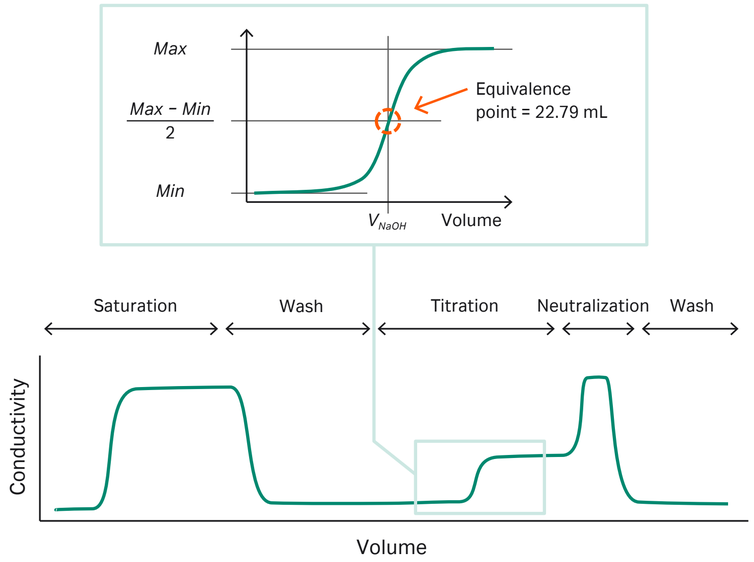
The HETP value can be retrieved automatically from the peak integration tool in UNICORN™ software.

Ionic capacity

The ionic capacity of the resin can be measured by on-column titration.

Phase	Eluent	Volume	Flowrate
Saturation	0.5 M HCl	5 CV	3 mL/min
Wash	Water	5 CV	3 mL/min
Titration	0.1 M NaOH	3 CV	0.4 mL/min
Neutralization	0.5 M HCl	0.7 CV	3 mL/min
Wash	Water	5 CV	3 mL/min

The volume needed to titrate the resin is taken as the equivalence point (50% breakthrough) from the resulting titration chromatogram.



Results and calculations

Example of raw data gathered from all experiments is presented below.

Experiment	Measured parameter	Measured value
System characterization	$V_{sys,UV}$	0.34 mL
	$V_{sys,cond}$	0.46 mL
Small tracer	V_{NaCl}	14.77 mL
Large tracer	$V_{Dextran}$	6.42 mL
	$HETP_{Dextran}$	0.031 cm
Ionic capacity	V_{NaOH}	22.79 mL

The raw data is then used to calculated all column parameters.

Parameter	Calculation
Total porosity	$\varepsilon_{tot} = \frac{V_{NaCl} - V_{sys,cond}}{CV} = \frac{14.77 - 0.46}{15.7} = 0.91 =$ **91%**
Interstitial porosity	$\varepsilon_{int} = \frac{V_{Dextran} - V_{sys,UV}}{CV} = \frac{6.42 - 0.34}{15.7} = 0.39 =$ **39%**
Axial dispersion	$D_{ax} = \frac{u_{linear} \times HETP_{Dextran}}{2 \times \varepsilon_{int}} = \frac{230 \times 0.031}{2 \times 0.39} = 9.1\ cm^2/h =$ **0.252 mm²/s**
Ionic capacity	$\Lambda_{backbone} = c_{NaOH} \times \frac{V_{NaOH} - V_{NaCl}}{CV \times (1 - \varepsilon_{tot})} = 0.1 \times \frac{22.79 - 14.77}{15.7 \times (1 - 0.92)} =$ **0.64 mmol/mL**

05

Summary of experiments

A mechanistic chromatography modeling workflow typically requires a model calibration step which includes system and column characterization, target molecule experiments, and parameter estimation. This section shows a high-level checklist on the recommended experiments that should typically be performed to calibrate the chromatography system and column for a mechanistic model.

5.1 System and column characterization experiments overview

Experiment name	Purpose	Suggested sample	Mobile phase	Loop volume	Flow rate	Column	Detector
Small tracer pulse, System	System dead volume calculation	1% Acetone + 0.8 M NaCl	0.4 M NaCl	1%–2% CV	Ideal process flow rate	Offline	UV, Conductivity
Small tracer pulse, Column	Adsorber total porosity calculation	0.8 M NaCl	0.4 M NaCl	1%–2% CV	Ideal process flow rate	Inline	Conductivity
Large tracer pulse, Column	Column interstitial porosity calculation	0.4 M NaCl + 10 g/L Dextran (2000 kDa)	0.4 M NaCl	1%–2% CV	230 cm/h or max resin flowrate	Inline	UV
Ionic capacity titration, Column	Adsorber ionic capacity (IEX only)	CEX: acid-base titration, *see* Section 2.6 for detailed description AEX: base-acid titration, *see* Section 2.6 for detailed description				Inline	Conductivity
Accessible surface area, Column	Accessible adsorber surface area determination (CPA model)	0.4 M NaCl + 10 g/L Dextran SEC standards	0.4 M NaCl	1%–2% CV	30 cm/h	Inline	RI

IEX = ion exchange chromatography

CEX = cation exchange chromatography

AEX = anion exchange chromatography

CPA = colloidal particle adsorption

RI = refractive index

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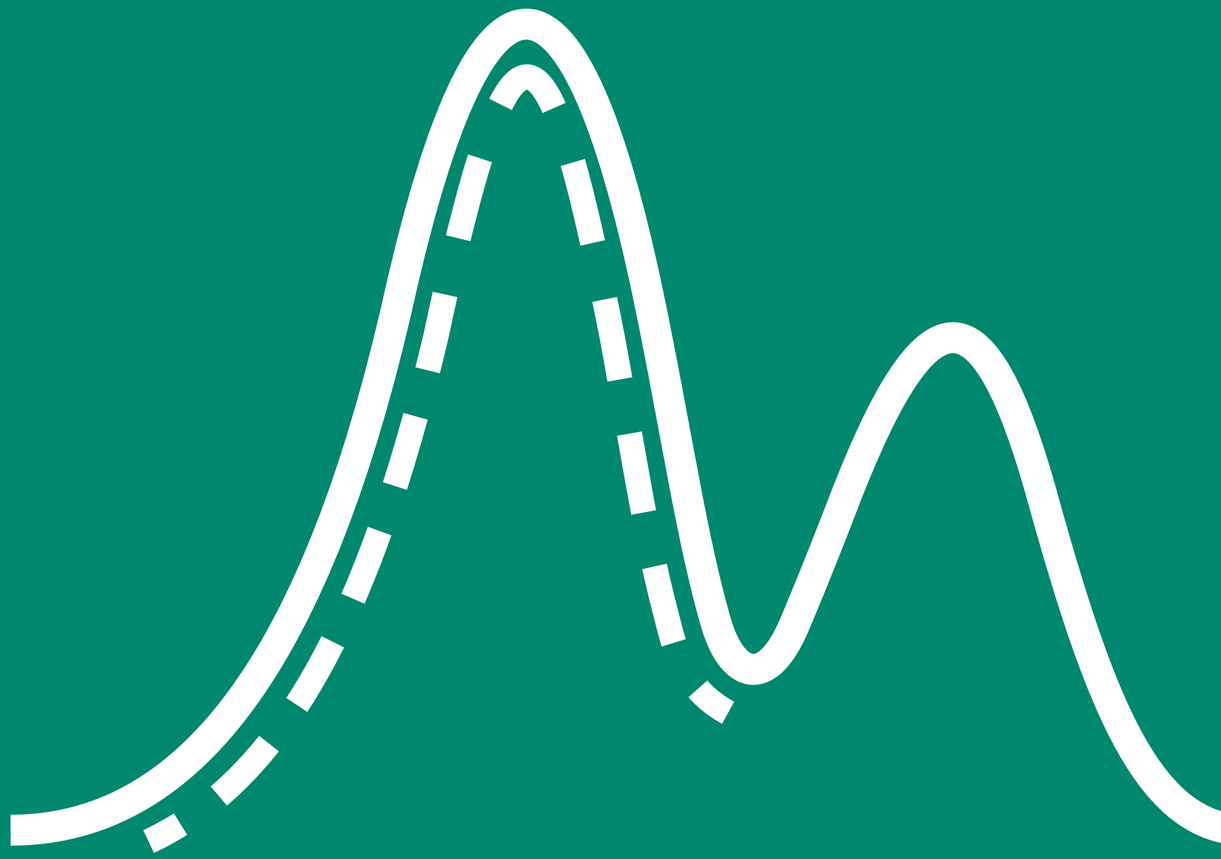

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