InPlace™ Chromatography Columns

VERDOT

- **Automated method-driven packing**
- **Scalable design, 20 cm - 200 cm**
- **Compatible with all resin types, including hydroxyapatite**
- **Ergonomic operation**
- **Compact footprint: ~50% lighter than other columns of similar capability**
- **Compact footprint: ~17% shorter than other columns of similar capability**
- **On-site packing support**
- **Custom designs to meet your process requirements**
- **21 CFR Part 11 and USP VI Compliant**
- **Complete documentation for regulatory submission**

Mobile Phase Distribution in VERDOT InPlace™ and EasyPack™ Chromatography Columns

Sébastien Lefebvre, VERDOT, Riom, France

Robust protein purification using chromatography requires a process capable of separating various kinds of molecules in a product load, at a high speed and high yield, independent from the column size—that is, in a scalable fashion.

The separation is tightly linked to the resolution of the column, and in turn, the resolution is tightly linked to the uniformity of the liquid distribution through the column. Therefore, the flow distribution is a principal feature of column design, especially at manufacturing scale.

The design of the distribution system must simultaneously address:

- Ideal fluid distribution
- Mechanical features for obtaining a watertight seal
- Mechanical resistance against resin compression as well as fluid flow

Figure 1. InPlace™ Chromatography Column

For the VERDOT InPlace™ (Figure 1) and EasyPack™ Columns, the distribution systems are identical and were designed using computational fluid dynamics (CFD) and confirmed by practical experimentation to evaluate performance.

Case Study

VERDOT executed the following studies to design and confirm fluid distribution performance:

- Theoretical: Fluid simulation using cfdesign®
- Theoretical: Mechanical stress calculation using Creo® Simulate (formally Pro/ENGINEER Mechanica) from PTC®
- Experimental: Practical test measuring Asymmetry and reduced HETP
- Experimental: Dye tests using Phenol Red

This case study describes the column distribution design and scalability with theoretical and practical performance across several column sizes.

Design of Column Distribution

For columns up to 100 cm diameter, a single central injection point is used for the mobile phase fluid path. The fluid from the single injection point must then spread as evenly as possible across the entire surface area of the column before proceeding downward into the bed.

The top adaptor is smaller than the column diameter to allow movement within the column; on the bottom distributor, the static filter (also called a frit) and distribution system have been mechanically designed to cover almost the entire column base surface. Therefore, fluid distribution in the top adaptor is sub-optimal compared to the fixed bottom adaptor. For that reason, the present study only focuses on the top adaptor, which is considered the worst-case scenario.

The design of the top flow adaptor (Figure 2) includes a deflector plate immediately below the injection point. The deflector plate is made of solid stainless steel. The flow is then distributed radially outward to the column wall as well as back underneath the plate, towards the center of the column.

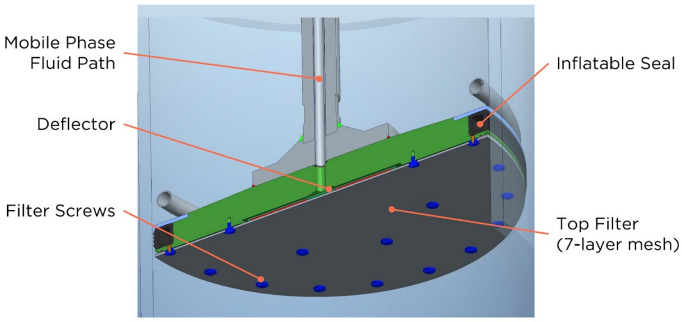

Figure 2. Top distributor design for column sizes 20-100 cm diameter (i.e., VERDOT D200-D1000 column sizes)

Under the deflector plate, the stainless steel mesh filter (standard porosity is 20 µm; custom porosity available on request) is mounted. Spacer bars on the surface of the piston keep the mesh filter from contacting the piston. The space between the filter and the piston allows for fluid distribution, promoting liquid flow to the walls of the column.

The outer edge of the Dual Flow Direction (DFD) top mesh filter (Figure 3) is specially designed for zero dead volume at the column walls, allowing for optimal fluid flow and distribution in this critical area.

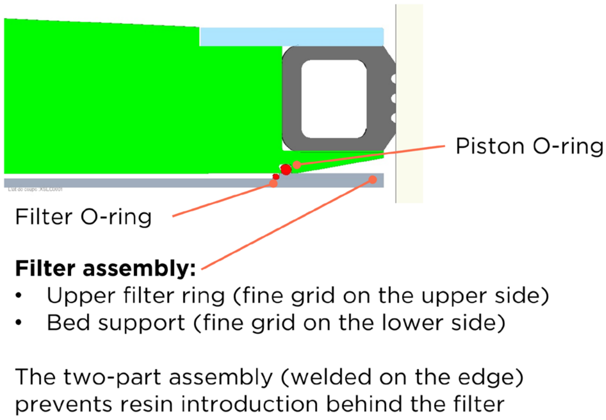

Figure 3. Detailed view of the top distributor outer edge with dual flow direction (DFD) filter resulting in zero dead space

For columns larger than 100 cm diameter, multiple points of injection (Figure 4) are required to obtain even flow distribution. The remainder of the top flow adaptor design is maintained, including spacer bars and DFD filters.

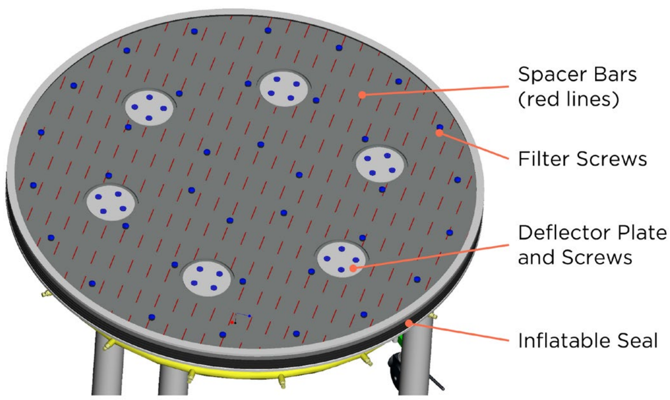

Figure 4. Top distributor design for VERDOT column sizes >100 cm diameter (i.e., VERDOT D1110-D2000 column sizes)

Fluid Simulation Using CFD Modeling

Several studies were executed, each with a different objective:

- *Permanent mode analysis:* The mobile phase injection was simulated at constant speed with stable conditions (i.e., pressure, permeability, fluid properties, bed packing, etc.) to analyze any difference in fluid distribution. Studies were first performed in 2D (shorter calculation time), followed by 3D.
- *Transition mode analysis:* A change of fluid was simulated, from a water-like fluid (Fluid A) to a process fluid (Fluid B) which had a viscosity and density different from water. Simulations and computational modeling illustrated the transition from Fluid A to Fluid B across different parts of the packed bed.

Simulations and computational modeling were performed for mid-size columns (≤100 cm diameter) with a single process injection point as well as for larger columns (>100 cm diameter) with several process injection points.

Simulation parameters

Fluidic Properties

For permanent mode analysis with stable conditions, water was the model fluid (Fluid A). For transition mode analysis, a change from water to a fluid with higher viscosity and density (Fluid B) was simulated. To accentuate the difference between the fluids, the model used a viscosity for Fluid B that was two times that of Fluid A and had a density of 1.1 g/mL (Table 1). This corresponds to a concentrated NaOH solution of 2.26 M at 13°C, or NaCl solution of 2.23 M at 2°C or to a dense IgG solution of 100 g/L at ambient temperature.

Table 1. Fluidic Comparison

	Density (g/mL)	Viscosity (Pa.s)
Fluid A (Water)	1.0	0.0001
Fluid B (Process Fluid)	1.1	0.0002

Packed Resin Bed Properties

The resin bed was simulated as a porous material, obeying the Darcy equation or, in the case of a bed made of rigid beads, using the Kozeny-Carman equation (Table 2).

Table 2. Resin Properties Considered

Resin	Extraparticle void fraction	Permeability (m^2)	Mean Particle Size (µm)
Bio-Rad Macro-Prep® High S/Q	0.302	7.86×10^{-13}	50
Bio-Rad CHT 40 Type 1 and 2	0.329	7.05×10^{-13}	40
Cytiva SP Sepharose™ Fast Flow	0.329	3.40×10^{-12}	88*
Cytiva Q Sepharose™ Fast Flow	0.329	1.91×10^{-12}	66*
Media model	0.329	7.62×10^{-13}	40

*Deduced from ΔP=f(linear speed) by Kozeny-Carman equation

Equations

Darcy's Law

$\delta p / \delta x_i = C.\mu.u_i$

Where C is the viscosity coefficient, x_i is coordinate in direction i, µ is the viscosity (Pa.s) and u_i is the velocity in the direction i

Kozeny-Carman (or Blake Kozeny) equation, for a porous bed

$\Delta P/L = \mu.V/\kappa$

With $1/\kappa = 180.(1-\varepsilon)^2 / (\varepsilon^3 . d_p^2)$

Where κ is the permeability in m^2, ε is the resin extraparticle void fraction, d_p is the mean particle size of the resin beads (in m), V is the superficial mobile phase velocity (in m/sec) and µ is the viscosity (Pa.s)

The interstitial mobile phase velocity (u) can be calculated from the superficial mobile phase velocity by the relation: $u = V / \varepsilon$

When a fluid change occurs, there is a diffusion of the molecules from the mobile phase into the static phase, obeying *Fick's Law* $J = -D.\delta c/\delta x$

Where J is the mass transfer flux (kg/m2/s), D is the diffusion coefficient (m^2/s) and $\delta c/\delta x$ is the concentration gradient ($kg/m^3/m$)

Limitations of the Model

This simple model neglects some physical phenomena such as:

- The velocity gradient around the particles (stagnant film) due to model limitations
- Mass transfer limitation at particle surfaces. The model considers small molecules, such as NaOH or NaCl, with a high mass transfer coefficient
- Pore diffusion limitation with a hindrance parameter depending on pore size. Again, the model considers small molecules with good pore diffusion
- Binding kinetics of target molecules and/or impurities. The model considers non-binding molecules such as NaCl or Phenol Red injected for packing evaluation (HETP, Asymmetry, visual)
- Wall effect contributions because for large scale columns (> 20 cm diameter) the wall effect is considered negligible

Analysis of the Fluid Dynamics in the Top Adapter Using Permanent Mode

Two column sizes were evaluated:

- 60 cm diameter column (D596), representative of VERDOT columns in the range of 20 cm to 100 cm with a single injection point
- 120 cm diameter column (D1200), representative of VERDOT columns with a diameter greater than 100 cm up to 200 cm with multiple injection points

Fluid distribution was modeled for the space between the piston and the filter, especially around the spacer bars that support the filter when the packed bed is compressed. The simulation used a superficial mobile phase velocity of 300 cm/h.

Figure 5 illustrates the mobile phase distribution in the chamber of the top adaptor for a D596 column. The vertical fluid dynamics moving down through the packed bed are not represented in this image.

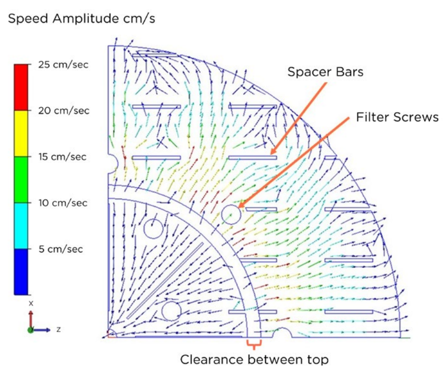

Figure 5. D596 column mobile phase distribution using permanent mode analysis

The mobile phase is injected in the central injection point (lower left corner, behind the distribution plate) and spreads out across the top surface of the deflector. Then, it passes through the clearance between the piston and deflector (demonstrated by the arc in the middle of the view). From this annular section, part of the flow extends to the column perimeter while the remainder of the flow covers the center. It is expected that the linear speed in the distribution channel is maximum at the clearance between the piston and the deflector. The diameter of the deflector must be precisely chosen so that the flow going to the column radius and flow going to the center are proportional to the surface distributed.

The mobile phase distribution is represented by the following equation:

$v(r) = V/(2 \cdot e)\ ((R^2+R_d^2)/r - r)$ for the flow extending from deflector to the column radius with:

$v(r)$: linear flow in the distribution channel at radius r, (m/sec)

V: the superficial mobile phase velocity (m/sec)
R: radius of the column (m)
R_d: radius of the deflector (m)
e: height of the distribution channel (m)

As expected, the model predicts that the linear speed decreases from the deflector edge to the column perimeter and from the deflector edge to the center. The spacer bars and the filter screws have minor interactions with flow distribution; however, the interactions are not significant.

For columns above 100 cm diameter with multiple injection points, the following view (Figure 6) shows the distribution of the mobile phase in the chamber of a 120 cm diameter piston. The fluidic veins moving down in the bed are not represented in this image.

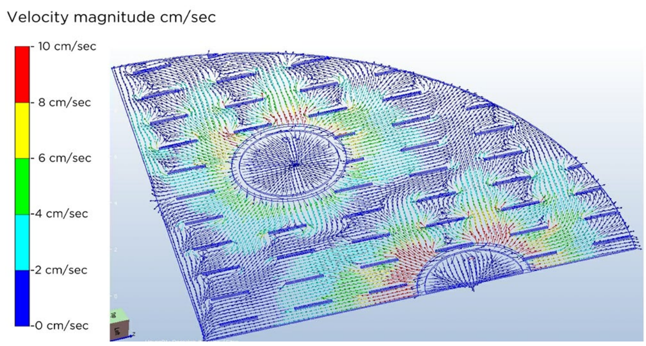

Figure 6. D1200 column mobile phase distribution using permanent mode analysis

As in the case of the D596 column, the linear flow in the distribution channel decreases as the flow extends to the column radius. As shown in Figure 6, the spacer bars have minor interactions with flow distribution, but due to the size and placement, the spacer bars do not prevent the entire surface from being irrigated. The speed gradient shows an elliptical fluid vein pattern rather than circular due to the pressure drop induced by the spacer bars. We can observe that the lower flow areas (in dark blue) are well distributed across the entire column surface: in the center, at half way between the injection points and at the outer edge of the column.

Distribution of the Mobile Phase in the Packed Bed

Because the diameter of the top adaptor is smaller than the column tube inner diameter to allow the adaptor to fit inside the tube, distribution heterogeneities observed at the column wall are unavoidable.

These heterogeneities are however very limited due to the dual flow distributor (DFD) design of the top filter, as the outer slope of the distributor allows fluidic veins to reach the inflatable seal bottom surface and the tube wall. The back pressure of the media also forces the fluidic veins to self-distribute evenly through the entire bed surface.

Figure 7 illustrates the distribution of the mobile phase in a packed bed at the outer edge of the top distributor with the DFD filter design.

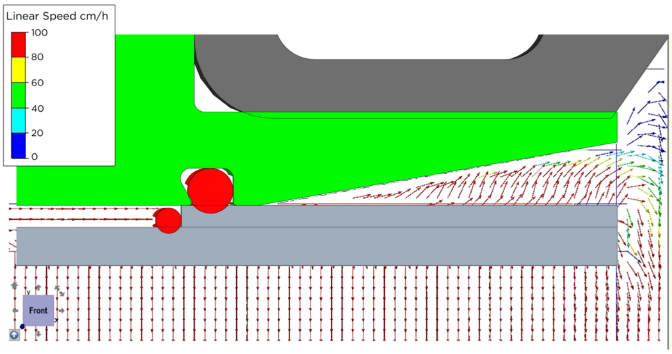

Figure 7. Dual flow distributor design of the top filter. Note flow distribution at the outer edge

Mechanical Resistance of the Filter From Compressed Packed Bed

A mechanical resistance analysis of the filter was performed to study the filter fittings, ie: screws and spacers. The filters are submitted to two kinds of resistance:

- Fluidic pressure, equivalent to the pressure drop of the filter, which is minimal (less than 20 mbar for a 20 µm filter subjected to water at 20 °C at 300 cm/h)
- Mechanical pressure resulting from resin compression, especially for semi-rigid beads. In this case, the packed bed acts as a spring with a stiffness equivalent to the Young modulus of the resin. Because the Young modulus can vary between different types of resin, we consider the most extreme scenario where the packed bed applies a strength against the filter equivalent to the maximum column design pressure, which can take place as a result of poor packing.

For ensuring that the bending of the filter between two spacers stays in an acceptable range, mechanical simulation with Creo® Simulate (formally Pro/ENGINEER Mechanica) from PTC® was performed. Figure 8 shows the maximum shear stress, and the potential deformation of the filter (magnified). The simulation is an extreme scenario where the filter is subjected to 6 bar as a result of the Young modulus (spring effect) from the resin bed.

All results of the simulation are within the acceptable levels (i.e., no permanent deformation). There is some shear stress observed around the filter screws and in some places of the outer edge of the filter flange that are away from the spacer bars. It was determined that the Von Mises stress is maximal at the edge of the spacer bars that can act as pinch points.

As the filter material is made of mesh and is not a uniform material, physical tests were necessary to determine if these potential pinch points are critical. The results of these tests indicate no evidence of unacceptable marking that could initiate corrosion.

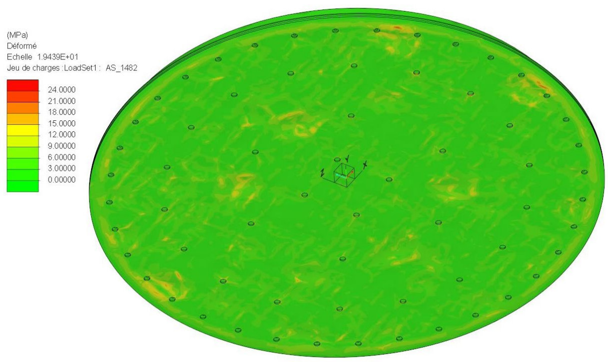

Figure 8. Simulation showing shear stress on the top filter due to the mechanical pressure from resin compression in a packed bed

Validation With Dye Tests

Dye tests consist of injecting a colored sample fluid in the column, preceded and followed by equilibration using the same buffer (without color) at constant speed. When the sample is near the middle of the bed height, the flow is stopped. The adaptor is then removed and an angular section of the bed is excavated in order to make the colored sample visible for examination from center to edge.

The advantage of this test is that it mimics the injection of a product sample in flow-through mode (no binding effect) and it makes visible any distribution abnormalities, provided that they appear in the cut. Dye tests are considered a good tool for qualitative analysis of a column design, in addition to more quantitative performance evaluations such as Asymmetry and HETP.

The following figures (Figures 9 and 10) present tests performed comparing the VERDOT InPlace Column to a competitor column with central slurry valve. Agarose-based resin was used in both cases. Both columns were 100 cm in diameter, which represents the largest VERDOT column diameter with a single injection point.

Figure 9. Dye test in 100 cm diameter VERDOT InPlace column shows an even and uniform dye front

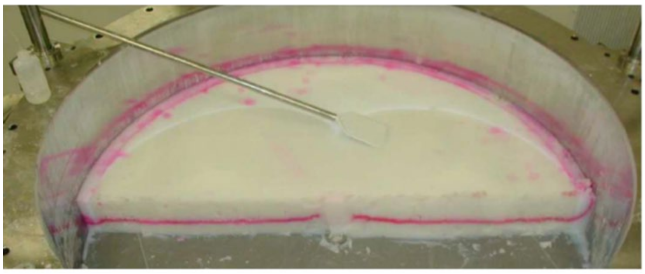

Figure 10. Competitor column with central slurry valve shows an even but non-uniform dye front

For the competitor column, it appears that the central valve creates a large blind spot in the distributor and prevents uniform distribution of the mobile phase in the center of the column.

For the InPlace Column, the heterogeneity resulting from the filter screws was also evaluated (Figure 11). The screws create a conical shadow of approximately 1.5 cm diameter x 2 cm height. This represents a volume of roughly 1.2 mL per screw, which is marginal compared to the sample volume. Therefore, the impact on HETP and asymmetry values is expected to be minimal.

Figure 11. Close-up view of dye front over a filter screw on the InPlace Column

HETP and Asymmetry Performance Tests

HETP and asymmetry tests consist of injecting a high salt sample into the column, preceded and followed by a low salt buffer at constant speed. Contrary to the dye test, the sample is fully eluted from the column in isocratic mode. A conductivity probe is used to detect the high salt peak eluted from the column and the HETP and asymmetry values are calculated. The advantage of this test is that it is a very well-known and routine test that can be performed quickly.

Buffer conditions may vary depending on specifications provided by the resin manufacturer. It should be noted that minor changes in the testing conditions can have a dramatic effect on the values obtained. Changes that can affect these values include flow rate, proximity of the salt injection to the column inlet, proximity of the conductivity detector to the column outlet, diameter of the hoses which connect the column to the fluid delivery system, etc. Standard acceptance criteria are generally defined as:

- Asymmetry: Ideally around 1, but can vary from one resin to another
- Reduced HETP (rHETP): Less than 5 for high resolution applications

Table 3. Various Resin Packing Studies resins using VERDOT InPlace columns

Resin	Column Diameter (mm)	Plate Count (N/m)	Asymmetry	rHETP
Tosoh Bioscience Ca++Pure-HA®	200	9252	1.31	2.8
Bio-Rad Macro-Prep® CM	280	4693	0.80	2.9
Cytiva SOURCE™ 30RPC	350	19450	1.03	1.7
Cytiva Sepharose™ 6 Fast Flow	400	3788	1.46	2.9
Cytiva Sephacryl™ S-200 HR	446	9802	0.83	2.0
Bio-Rad CHT 40 Type 1 and 2	446	11570	1.12	2.1
Thermo Scientific™ POROS™ XS	800	9875	0.80	2.0
Bio-Rad Nuvia™ HR-S	800	7150	0.93	2.8
Cytiva DEAE Sepharose™ Fast Flow	1200	4400	0.96	2.2
Experimental polyacrylamide resin, monodisperse, 59µm	1600	4564	1.04	2.6

All packing results are well within the acceptable range (Table 3), demonstrating that customers can achieve optimal performance for every type of resin using the InPlace and EasyPack columns. For additional guidance, an experienced team of VERDOT experts can provide onsite packing support.

Specifications

Nominal ID (cm)	Packed Volume with 20 cm bed height (L)	Maximum pressure at 30°C (Bar)		
		Calibrated Borosilicate Glass	Stainless Steel 316L	Acrylic
20	6.3 L	4.7	6.0	6.0
25	9.8 L	3.8	6.0	6.0
30	14.1 L	3.6	6.0	6.0
35	19.2 L	---	6.0	6.0
40	25.1 L	---	6.0	6.0
45	31.8 L	2.4	6.0	6.0
50	39.3 L	---	6.0	6.0
60	55.9 L	---	6.0	6.0
70	77 L	---	3.0	3.0
80	100.5 L	---	3.0	3.0
100-200	157.1-628 L	---	3.0	3.0

Dimensions

Nominal ID (cm)	Dimensions (W x D mm)	Working height at 20 cm bed height*	Total column weight empty (kg)*
20	694 x 682 mm	1269±20 mm	136
25	744 x 732 mm	1290±20 mm	184
30	790 x 647 mm	1599±20 mm	220
35	844 x 814 mm	1599±20 mm	271
40	856 x 856 mm	1587±20 mm	317
45	906 x 906 mm	1587±20 mm	358
60	1085 x 1051 mm	1725±20 mm	629
70	970 x 880 mm	1725±20 mm	725
80	1350 x 1350 mm	1770±20 mm	1143
100	1350 x 1350 mm	1817±20 mm	1405
120	1767 x 1610 mm	2350±20 mm	2672
140	1982 x 1982 mm	2350±20 mm	3885
160	2182 x 2182 mm	2350±20 mm	4800
200	2430 x 2430 mm	2822±20 mm	7400
Intermediate column sizes available. Inquire for more information.			

*Dimensions based on InPlace Chromatography Column with a standard acrylic tube of 60 cm height