

Bioprocess Chromatography

CHROMATOGRAPHY forms the heart of the purification train for many bioprocess facilities. In a typical train, three or four chromatography steps are used to remove impurities and side products from the bulk product that is harvested from bioreactors. Each step employs a specific resin that preferentially bonds to either the product or an impurity, or exchanges ions with the product broth, to sequentially purify and concentrate the desired product molecules. This article provides information that will help engineers understand how the chromatography columns are sized. It gives some rules of thumb that give a range for sizing the operating buffers that the columns require. And it gives a typical chromatography piping and instrumentation diagram.

Scientists usually develop the purification process in a lab or pilot plant. They pass off the design of the production facility to process engineers who need to provide an appropriate space, facility features, buffer supply, and waste handling. The resins used in chromatography columns, especially Protein A resin, are expensive so the well-informed engineer can greatly influence the cost and complexity of the facility by applying sound principles and engaging the scientists and equipment vendors in meaningful debates.



Engineers: Cost and complexity can be influenced by applying sound principles and engaging with scientists and equipment vendors

Columns are sized from the quantity of material that will be captured. This establishes the amount of resin that is needed, and the column is dimensioned to hold the resin. Monoclonal antibodies typically load on the resin in the range of 15-60 g/L. The loading varies with the loading rate (a higher velocity decreases the capacity), the manufacturer of the resin, and the amount of breakthrough that is tolerated. Face velocity for loading the column typically ranges from 400-1,600 cm/h. The broth harvested from the bioreactor is usually subdivided and the column purifies the harvest in multiple batches that are called “cycles”.

Chromatography columns are cylindrical. The resin beads are packed into the columns according to a standard protocol; at manufacturing scale the depth of the bed may range from about 10–30 cm but is usually from 20-25 cm. We can now make an estimate for sizing a Protein A column for purifying monoclonal antibodies.

Given: 5,000 L harvest with 4 g/L titer, estimate the size for the Protein A chromatography column.

Answer: There are 20,000 g of monoclonal antibody in the harvest. The volume of resin should be in the range of 20,000/15 to 20,000/60 or 330 to 1,330 L. Calculate column diameters that equate to these volumes, using a bed depth of 20 cm for cycles from 1 to 16. See *Table 1*. By inspection, a column diameter of 85 cm (but round to a standard column size, say 80 cm) seems reasonable. Using a loading rate of 800 cm/h, calculate the volumetric flow of 67 L/min to load the column. This gives the total time for loading the column as 75 minutes, or about 9 minutes for each of 8 cycles.

VOLUME, L	CYCLES				
	1	2	4	8	16
300	138	98	69	49	35
600	195	138	98	69	49
900	239	169	120	85	60
1,200	276	195	138	98	69

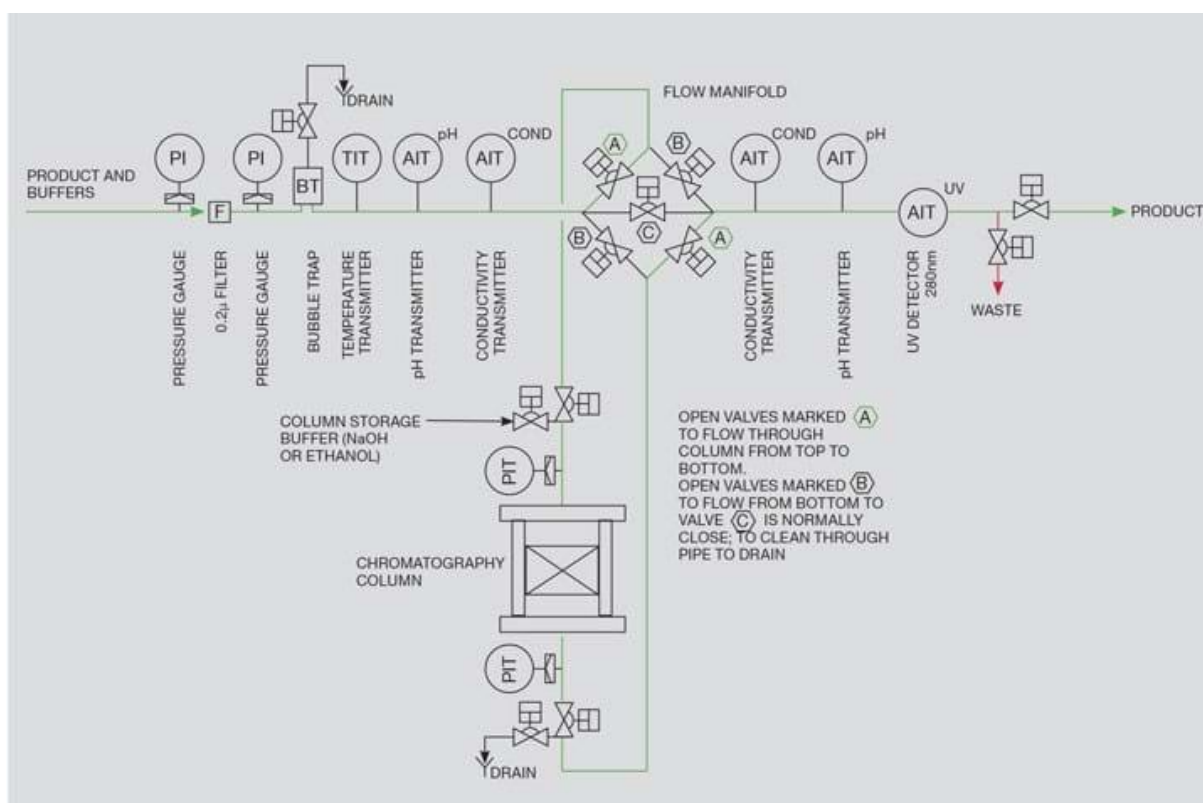
Table 1: Column Diameter (cm) for a 20 cm bed depth

The column operation requires at least five distinct steps (representative buffers listed):

- Equilibrate using about 5 column volumes (CV) of 25 mM phosphate buffer, pH 7.5.
- Load the broth by pumping at 400-1600 cm/L face velocity as discussed above.
- Wash using 5-10 CV of a 1 M tris-acetate buffer, pH 6.0.
- Elute the protein using 5-10 CV of 55 mM acetic acid buffer, pH 3.2.
- Regenerate with 6 CV 1 M NaCl solution.

Between uses, the column may be sanitised with 4 CV of 1 M NaOH solution, and stored in 0.1 M NaOH solution. For the example problem, using a rate of 1,200 cm/L velocity for steps 1, 2, 4, and 5 and assuming 8 cycles to purify 5,000 L, calculate a total process time of about 5 hours. Four different buffers are used at volumes from 4,000-6,000 L (total 21,000 L of buffers).

The operating steps flow downward or upward through the column, and the piping arrangement allows either direction. Figure 1 shows the instruments and valving that typically surround a chromatography column. The buffer formulation and holding tanks and their distribution piping are not depicted.



You should now have a conceptual feel for the primary chromatographic purification step in a bioprocess, and information that approximates the equipment sizes and capacities that are needed. This isn't an exact science, the biological processes and equipment are constantly being improved, and contingencies are always required. The latest advances in chromatography involve continuously operated equipment, multicolumn and moving bed chromatography. As titers improve (currently around 5 g/L but expected to increase to 10-20 g/L) you can calculate the impact on chromatography purification, and see the pressing need for these technology advances.

This is the third in a series that provides practical insights into on-the-job problems. To read more, visit the series hub at <https://www.thechemicalengineer.com/tags/rules-of-thumb>