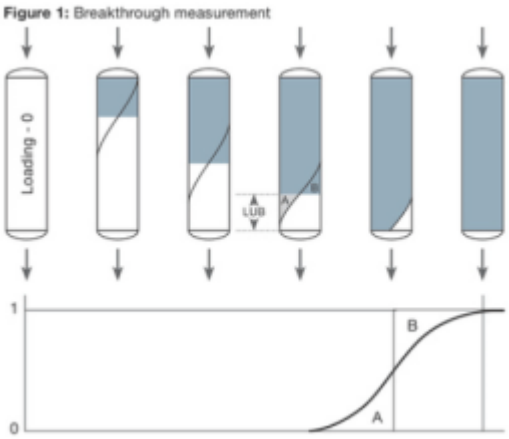
Do you ever worry about your column's loadability? Do you find yourself Googling, "How much am I able to load onto XYZ phase?" Ever fret over this issue so much you can't get a wink of sleep at night?

Well for preparative chromatographers, this might be the case. Column loadability directly influences the throughput and heavily influences process cost, creating what can be a major issue.



For those lucky biochromatographers who typically work with ion-exchange phases, they can anticipate a concrete answer for how much to load. Yet, when it comes to reversed phase and normal phase chromatography there is no simple answer.

As it turns out, there are several ways to go about measuring column loadability.

One way you can determine dynamic loading capacities is by breakthrough measurements. A solution of known concentration is continuously injected at the column head, then time is measured until the solute substance reaches the detector which is indicative of column/ phase saturation.

This dynamic loading capacity is smaller than the static loading capacity of the phase and is dependent on the flow rate. The faster the flow rate, the lower the loadability. Typically, 10-25% of the maximum dynamic loading capacity can be injected onto the column for the chromatographic separation.

Another way to measure loadability is by monitoring certain chromatographic parameters such as retention time, peak width and the plate height. A column is said to be overloaded when the parameter has changed by +/- 10% from its original value determined during the

analytical loading studies.

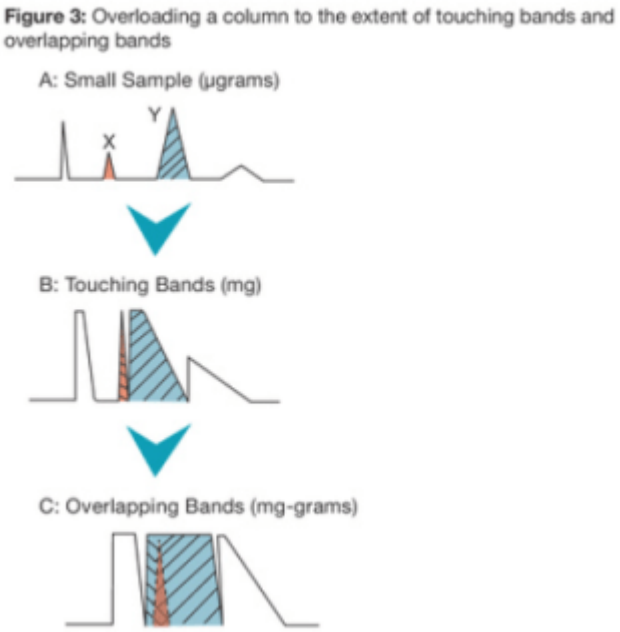


Figure 3: Overloading a column to the extent of touching bands and overlapping bands

However, these loading measurements are performed with a single compound and are of limited value in estimating the loadability of a new mixture. The only practical way to determine the exact phase loadability is to run a loading study under optimized analytical conditions and then to increase the concentration load stepwise taking fractions across the peak area of interest and analyze the collected fractions for purity.

Here at Phenomenex, Dr. J Preston came across a similar issue.

“I worked on a reverse phase purification project that had some loading issues. The first loading study used larger and larger injection volumes of the sample dissolved in a typical diluent. Fairly low loads demonstrated an anti-Langmuir isotherm where the peaks exhibited fronting that increased with larger injection volumes. I changed the sample diluent to a strong solvent and was able to prepare a more concentrated sample.”

It is typically recommended to heavily overload the column because so-called non-linear effects like displacement effects and tag-along effects show up at such high loadings, may

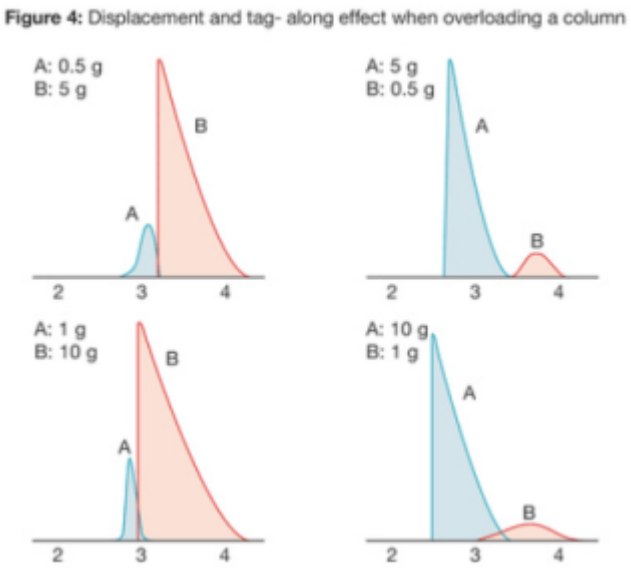


Figure 4: Displacement and tag- along effect when overloading a column

work in favor of the separation.

Dr. Preston continued saying, “another loading study was performed with smaller injection volumes but more sample being loaded onto the column and the peak shapes were significantly improved. The overall process had significantly higher throughput by using the stronger diluent and injecting smaller volumes.”

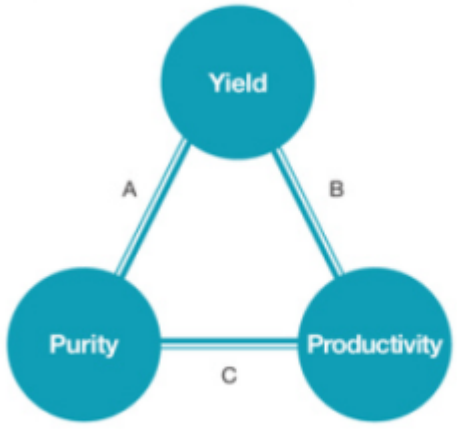
However, a simple rule of thumb when it comes to loadability is that the minimal specific loadability should be 1% for a properly optimized reversed phase-HPLC process. This is expressed as g of crude per 100g of stationary phase. In some cases, chromatographers using high surface area media with excellent mechanical strength have reported loadabilities exceeding even 5% which present tremendous economy of scale. It has been found that normal phase methods exhibit higher loadabilities (5-10 fold) compared to reversed phase methods.

But we can’t stress enough that such results do not offer a hard rule because the key parameters—productivity, yield, and purity of chromatographic separation are interdependent and cannot be maximized all at a time.

A true loadability of a media phase is rather a compromise depending on the purification goals and on which of the three parameters is most focused on.

We will leave you with this last thought though; it is exceptionally important to define the purification goals first and then complete the fraction analysis at several loading conditions to find the optimal conditions to achieve your purification goal.

Figure 5: What are the purification goals?



A: Low/moderate overloading: touching bands; B: Heavy overloading, low efficiency; C: Overloading, narrow target fraction

For a more detailed look at loadability or any questions you might have, please visit our Purify Newsletter for the full article, “How Much Can I Load?” or reach out to our Technical Support team.

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