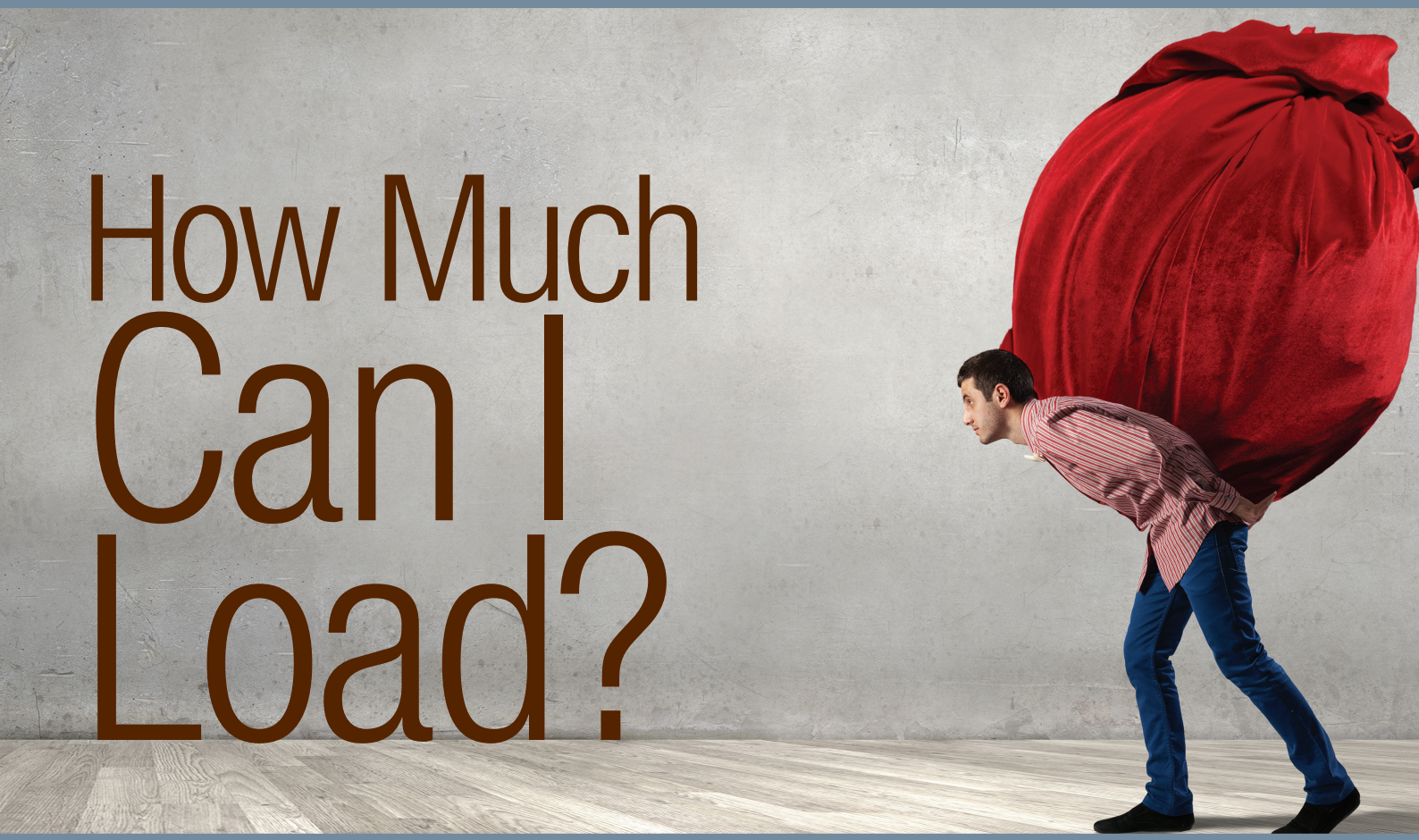
How Much Can I Load?



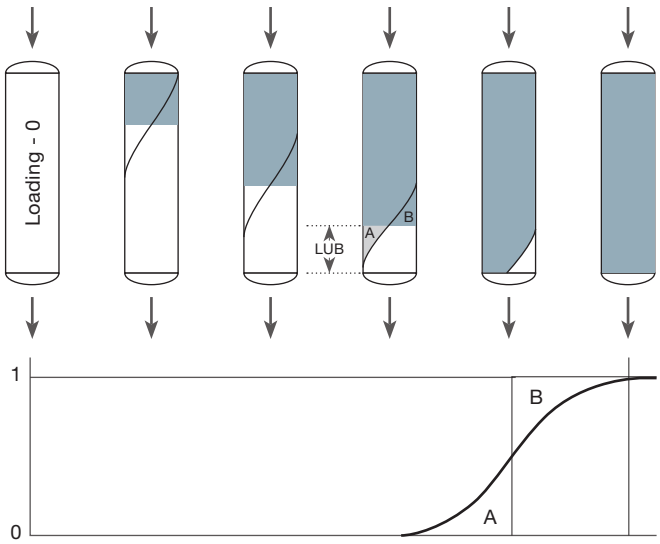
Prep Chromatography Loading for Maximum Recoveries and Yields

Column loadability is a major issue for preparative chromatographers because it directly influences the throughput and heavily influences process cost. A common question for preparative chemists and chromatographers is “How much am I able to load onto XYZ phase?” This is especially true for biochromatographers who typically work with ion-exchange phases and anticipate a concrete answer, such as the ion capacity (in meq/g). Unfortunately when it comes to reversed phase and normal phase chromatography there is no simple answer to this apparently easy question.

There are several ways to measure column loadability. One way is by dynamic loading capacities determined by break-through measurements. To find the dynamic loading capacity, a solution of known concentration is continuously injected at the column head. 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.

Figure 1: Breakthrough measurement



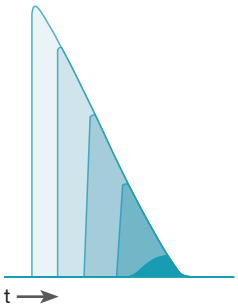
The dynamic loading capacity can be calculated according to:

$$L = F \times C \times Tb / CV$$

(F = Flow rate, C = Feed concentration, Tb = Breakthrough time, CV = Column volume)

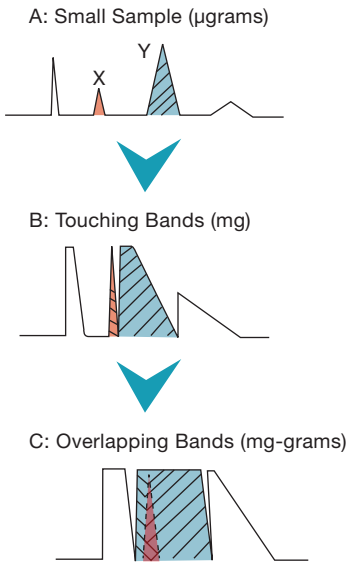
Loadability can also be measured by monitoring certain chromatographic parameters such as retention time, peak width and the plate height. A column is said to be overloaded when the parameter has changed by +/- 10 % from its original value determined during the analytical loading studies.

Figure 2: Change of retention time and peak width with increasing load



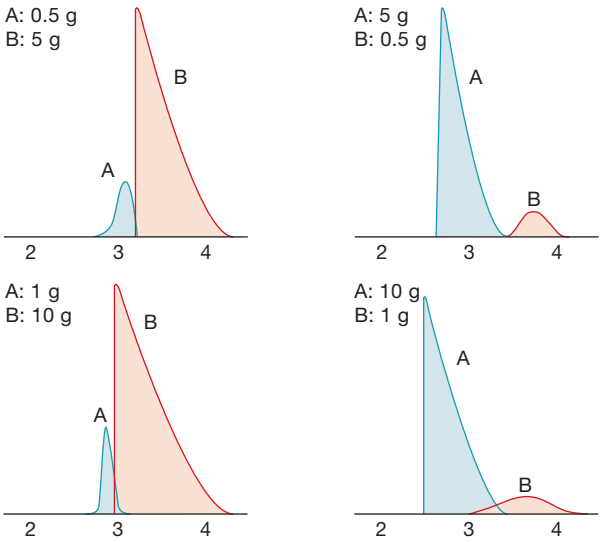
However, all of these loading measurements are performed with a single (PURIFY) compound and are of limited value in estimating the loadability of a new mixture. The only practical way to determine the exact phase loadability is to run a loading study under optimized analytical conditions (found by method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

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



It is always recommended to heavily overload the column because so-called non-linear effects like displacement effects and tag-along effects, which show up at such high loadings may work in favor of the separation.

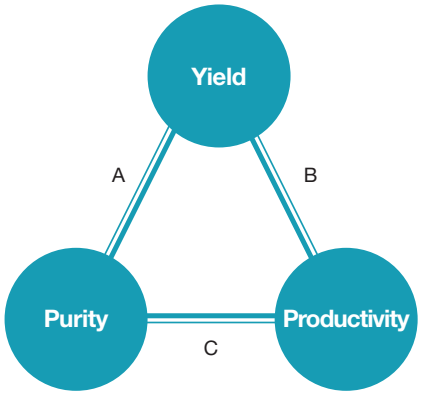
Figure 4: Displacement and tag- along effect when overloading a column



There is a simple loading rule of thumb. For a properly optimized reversed phase-HPLC process, the minimal specific loadability should be 1 %. 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 again, such results do not offer a hard rule for loadability because the key parameters, productivity, yield, and purity of a chromatographic separation are interdependent and cannot be maximized all at a time.

Figure 5: What are the purification goals?



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

The true loadability of a media phase is rather a compromise depending on the purification goals and on which (one or two) of the three parameters is most focused on.

It is therefore important to define the purification goals first and then complete the fraction analysis at several loading conditions to find the answer as to what conditions (load, fraction collection points) are optimal to achieve the purification goal.