Prep Chromatography Loading For Maximum Recoveries and Yields

Loadability is a major issue for preparative chromatographers because it directly influences the throughput and thereby the process cost. We are frequently asked by our customers: “How much can I load onto your XYZ phase”. Especially biochromatographers who are used to working with ion exchange phases expect a concrete figure as an answer, such as the ion capacity (in meq/g). Unfortunately when it comes to RP and NP chromatography there is no simple answer to this apparently easy question.

There are several ways how to measure loadability. Dynamic loading capacities can be determined by break-through measurements. A solution of known concentration is continuously injected at the column head and the time is measured until the solute substance reaches the detector which is indicative for the saturation of the phase in the column.

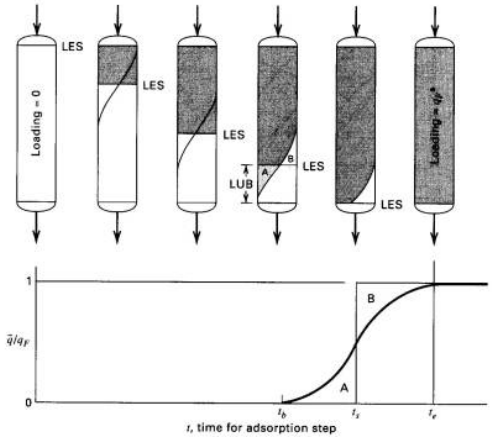

Fig 1: Breakthrough measurement

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)

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

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

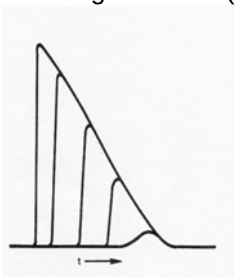

Fig 2: Change of retention time and peak width with increasing load

Key words: Loading Capacity, Overloading, Breakthrough

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However, all these measurements are performed with a single (pure) compound and do not take into account needed resolution between other eluting compounds. Such measurements may, however, be useful concerning the appropriate pore size of the stationary phase as they allow comparative studies of the accessible surface area of different phases for the compound of interest.

The only practical way to determine the loadability relevant under separation conditions is to run the separation under the best conditions found by method development under analytical conditions and then to increase the load stepwise, to take fractions across the peak area of interest and to analyze the collected fractions.

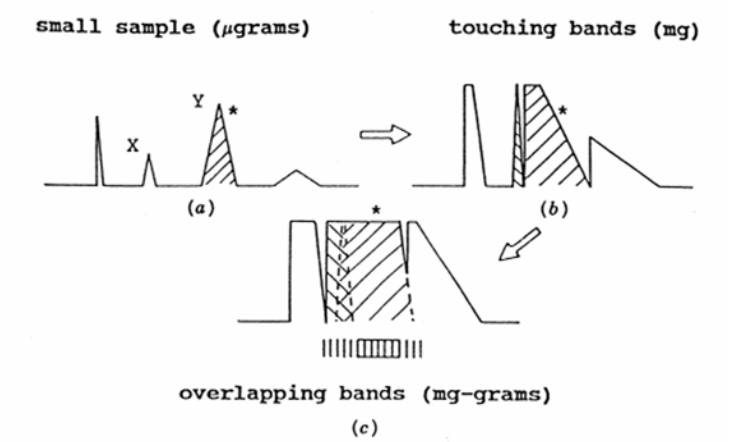

Fig. 3: overloading a column to the extent of touching bands and overlapping bands

It is always recommended to also try to heavily overload a column because so-called non-linear effects – displacement effects and tag-along effects - which will only show up at such high loadings may work in favor (or not) of the separation.

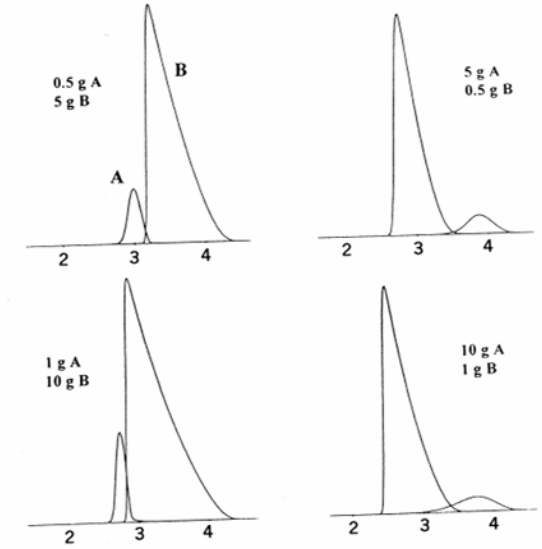

Fig. 4: Displacement and Tag along effect when overloading a column

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There is a rule of thumb saying that for a decently optimized RP-HPLC process the minimal specific loadability (expressed as g of crude per 100 g of stationary phase) should be 1%. A limited number of cases have reported loadabilities exceeding even 5%. NP methods typically exhibit higher loadabilities (5 – 10 fold) compared to RP methods.

But again such experiments do not result in a hard figure for the loadability because the key parameters productivity, yield and purity of a chromatographic separation are interdependent and cannot be maximized all at a time. The loadability a chromatographer will choose is rather a compromise depending on the purification goals and on which (one or two) of the three parameters he will put most focus. It is therefore important to define the purification goals first and then the fraction analysis at several loading conditions will give an answer as to what conditions (load, fraction collection points) are optimal to achieve them.

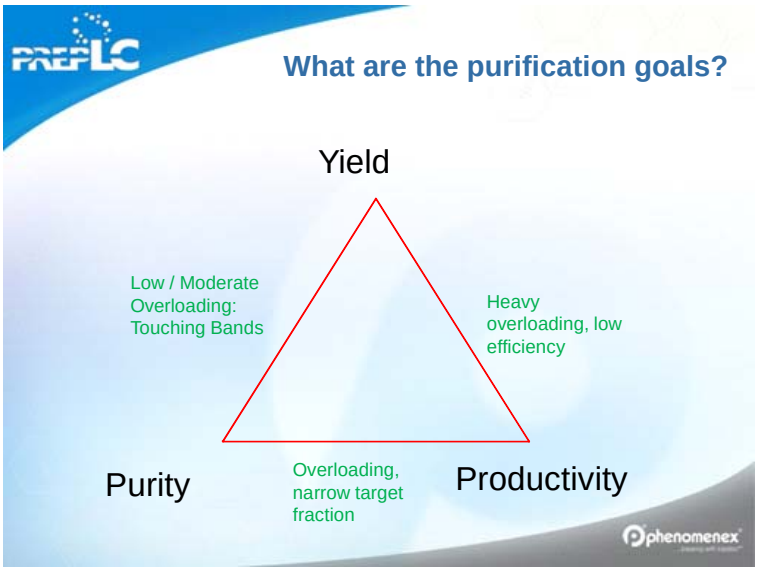

Fig 5: choice of operating conditions depending on purification goals

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