Loadability – what does it mean to preparative chromatographers?

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Introduction

The term loadability is often used in context with preparative chromatography. The promise of high loadability is an ever returning argument used by the suppliers of stationary phases.

While the advantages of high loading capacity in preparative chromatography is unchallengeable, it becomes however apparent, that many different definitions are used to describe this important feature of a chromatographic sorbent. Consequently, an unbiased comparison of the loadability for different sorbents is seldom found. In this study we show that loadability in $mg_{crude}/g_{packing}$ or mg_{crude}/mL_{column} barely is one factor contributing to the obtained productivity. In order to achieve high productivity, the sorbent needs to exhibit high loadability, adequate selectivity between the target compound and its neighboring impurities, as well as optimized retention time. Only if all these parameters are optimized, the preparative separation will lead to the required purification effect at the highest possible productivity.

By means of two preparative HPLC separations it is exemplified, that the term loadability is not a general characteristic of a stationary phase, but is highly dependent upon the actual application.

Touching bands or going further……..

Chiral separations have become a crucial part in drug discovery, and the need to put forward 100 mg quantities of pure enantiomers is a common objective for synthetic chemists.

Working with touching bands is a very convenient way of overloading, as no fraction analysis is required. The loading is increased, until the peaks touch each other due to mass overloading. As there is no overlap between the peaks, 100% product purity will be achieved with 100% recovery (Fig.1). The loading is highly dependent upon the selectivity α. Based on the band broadening of the individual enantiomer, one would expect a severe overlapping if the loading is increased further (Fig. 2), and therewith poor recovery for a pure product fraction. However, due to competition for interaction sites, the lesser retained enantiomer is displaced, meaning that it is eluting earlier than when injected individually (Fig. 3). This behavior results in a separation that is better than expected.

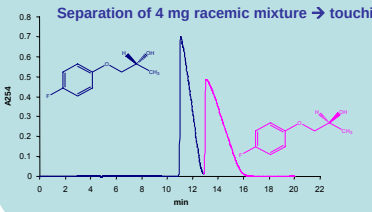


Figure 1: Separation of 4 mg racemic 4-fluorophenoxypropanol on Kromasil CelluCoat 10µm (4.6x250 mm), mobile phase: Heptane / IPA 95/5 (v/v), flow rate: 0.7 mL/min

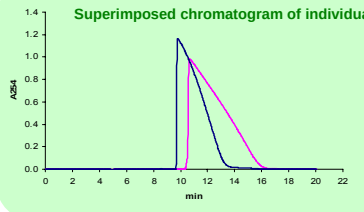


Figure 2: Overlaid chromatograms of individual injections of 15 mg R- resp. S-4-fluorophenoxypropanol . Same experimental conditions as in Figure 1.

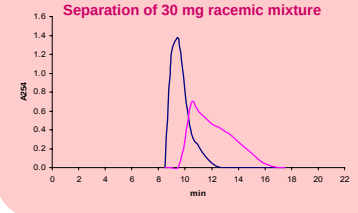


Figure 3: Separation of 30 mg racemic 4-fluorophenoxypropanol. Same experimental conditions as in Figure 1. Elution profile is based on fraction analysis.

As can be seen in in Table 1, the productivity increases drastically when utilizing the loadability of the stationary phase to the extent, where the two enantiomers compete for interaction with the chiral stationary phase. As is shown in this example, the productivity can often be increased by a factor of 10 when moving from touching band separations to competitive interaction overloading.

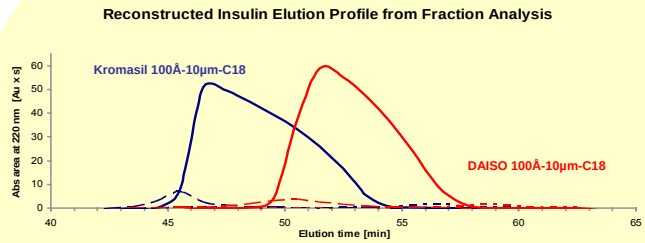
Table 1: Productivity comparison between touching bands and competitive interaction overloading

	Purity [%]	Recovery [%]	Productivity [$mg_{purified} \cdot mL_{col}^{-1} \cdot h^{-1}$]
4 mg injection	100	100	**1.7**
30 mg injection	92.4	82.0	**9.9**
30 mg injection	100	62.0	**7.5**

Peptide purification – Self displacement chromatography

In industrial scale preparative HPLC, the productivity demand is pronounced, as the equipment depreciation, solvent and labor costs are burdening the separation costs. Only high productivity ($g \cdot kg^{-1} \cdot h^{-1}$) will lead to acceptable total separation costs ($/$kg_{purified}$). Thus, it is always attempted to maximize the productivity, for a given purity requirement. The highest possible relative loading (mg_{crude}/mL_{column}) is often obtained when a self-displacement effect can be utilized. This is the case when the local concentrations of the components to be purified are not negligible compared to the capacity of the stationary phase, they compete for interaction with this phase[1]. When the bands of two components of the feed interfere, the more retained one tends to force the desorption of the lesser retained. The apparent retention of the lesser retained component decreases as it is displaced by the more retained one.

As is shown in Fig. 4, the band width itself is not indicative for the separation power of the packing material. For a 30 mg injection of crude human insulin on a 4.6x250 mm column, Kromasil 100Å-10-C18 renders a wider peak than DAISO 100Å-10µm-C18. Thus, it can be concluded, that the loadability itself is lower for Kromasil than for DAISO. However, Kromasil is able to displace the front impurity to a much larger extent, resulting in higher recovery and productivity than DAISO.



Column: 100Å-10µ-C18 (4.6x250 mm)
Feed: 900 µL of human recombinant insulin (Sigma-Aldrich). Sample concentration 33.3 mg/mL
Mobile phase: 200mM NH_4Ac pH 4.0 / EtOH
Gradient: 0-5 min: 10% EtOH, 5-67 min: 30-38% EtOH
Flow rate: 0.75 mL/min

	Peak width [min]	Purity [%]	Recovery [%]	Productivity [$mg_{purified} \cdot mL_{col}^{-1} \cdot h^{-1}$]
Kromasil 100Å-10µm-C18	9.7	98.6	76.5	**4.9**
DAISO 100Å-10µm-C18	7.8	98.0	59.2	**3.4**

* Cycle time for Kromasil: 62 min, for DAISO 70 min (including column wash and re-equilibration)

Figure 4: Comparison of loadability and productivity of human insulin with different C18 packing materials

Discussion and Conclusion

The two examples of preparative HPLC show that the true potential of a stationary phase can only be assessed when tested under genuine conditions. Therefore, the loadability of stationary phases has to be studied experimentally for every specific application, with a clear focus on maximizing the productivity while fulfilling the purity requirements.

Furthermore, it was shown, that competitive interaction overloading leads to significantly higher productivity than when working with the touching band method, despite the obvious loss in recovery.

Reference

[1] G. Guiochon et. al.; Fundamentals of Preparative and Non-Linear Chromatography, 2nd Edition, Elsevier Academic Press, Amsterdam, 2006