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The counterintuitive role of extra-column volume in the determination of column efficiency and scaling of chromatographic processes

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ABSTRACT

In industrial liquid separation processes chromatography often has a key function in the optimization of yield and purity. For the design of an industrial system, chromatographic processes are generally simulated using mathematical models, tested and optimized at laboratory level, and then scaled up to pilot and subsequently industrial scale. To describe the system, experimental data and model data need to be fitted and extra column contribution must be determined. This paper describes the influence of extra-column volume on overall separation efficiency for lab scale and its impact on the design of large scale systems.

Measurement of extra-column contribution was investigated in terms of mean retention time and variance using two different methods the commonly used zero dead volume connector and as an alternative the zero length column. Further a technique is presented to estimate extra-column contribution to band broadening for different injection volumes, velocities, and tracers based on representative measurements.

When scaling up, often contribution of extra-column volume from laboratory equipment is neglected assuming to be on the safe side, however column efficiency is often lower than efficiency measured for the entire chromatographic system. Relation between system efficiency and column efficiency was investigated using laboratory data and the lumped kinetic model. Depending on the ratio of extra-column volume to retention volume in the system, deduced column efficiency was up to 20% smaller than overall system efficiency. This ratio revealed the misleading nature of the term efficiency loss, when describing influence of extra-column volume on column efficiency. A scheme, which relates the relative variance of the system to the relative extra-column volume, provided an assessment of under- or overestimation of column efficiency. In this article it is shown how scaling up a system based on laboratory data, where extra-column volume contribution is not accounted for, may severely overestimate column efficiency. This overestimation results in underestimated column dimensions at pilot and industrial scale, and hence underperformance of the industrial system.

1. Introduction

1.1. Extra-column contribution in chromatography

Chromatographic separation methods are common practice in most analytical and preparative separation applications. Except for thin layer chromatography, all chromatographic methods share inherent construction of one or several columns connected to equipment such as detectors and valves via tubing/piping and connectors. Scaling up chromatographic processes starts with the right interpretation of laboratory experiments. When analyzing

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any chromatogram it is important to keep in mind that mobile phase and analytes pass not only through the column but through the entire system, every part of equipment in the flow path between sample injection and detection including the column. Each part adds to overall retention time and band broadening (expressed in terms of variance). If the column is taken out, then the resulting chromatogram shows the extra-column contribution to retention time and variance [1–3]. It is not possible to show the chromatogram of the column alone. Only by accounting for extra-column contribution in the measured system, properties of the column can be deduced. If the contribution to band broadening of the chromatographic column is not separated from the contribution of the extra-column volume, then estimation of column dimensions during development and scaling up may be subject to large errors.

The challenge of determining the influence of extra-column volume to band broadening and role of individual equipment parts before and after the column was first discussed in 1966 for gas chromatography by Sternberg [1]. The concept of extra-column band broadening was theoretically applied to analytical liquid chromatography in 1975 [4] and practically investigated the following year for contribution of injection system, detectors, connectors and guard columns [2]. These articles demonstrate an increase in peak variance caused by equipment parts before and after the column and argue for a minimization of extra-column band broadening in order to utilize the full separation efficiency of the column. More recent literature is primarily concerned with minimization of extra-column volume and optimization of flow channels to reduce extra-column variance [2,5–7] as well as modelling the effect of each part [7–11].

Column efficiency, describing band broadening of an injected pulse migrating along the column, is one of the most important scaling parameters [12]. When scaling up a system, column efficiency should be kept constant [13] or must be accounted for. Several publications state that efficiency of the column is almost or always higher than efficiency of the entire system [8,14,15]. Most publications reviewed for this work do not specify retention time and variance of column and of extra-column volume, so the reader cannot deduce column performance within that specific system e.g. [6,15,16]. Preliminary experiments indicated that efficiency, defined on the basis of retention time and variance, was not always reduced when extra-column contribution was taken into account. A system efficiency larger than the column efficiency has to our knowledge not been described before. It opposes the intuitive term “efficiency loss”, which is associated with extra-column volume [5].

This work aimed at better understanding efficiency loss due to extra-column volume and extra-column contribution to band broadening. Elution profiles of pulse injections were investigated for the calculated efficiency before and after accounting for extra-column volume, and the resulting efficiency loss was analyzed. Two methods for measurement of extra-column contribution were compared: a zero dead volume connector (ZDV) and the alternative “zero length column”. The latter retains all construction parts found in the resin filled column (flow distributors, frits, filters, etc.), except for the space the resin occupies in a regular column as well as the resin itself, and therefore gives a more accurate representation of extra-column contribution to retention time and variance than the commonly used ZDV. In addition, the influence of injection volume, mobile phase velocity and tracer molecule on extra-column contribution was investigated to enable accurate estimation of extra-column variance for different experimental settings. This work will show that in many cases system efficiency will be higher than column efficiency after correction for extra-column contribution.

A guide to estimate under- or overestimation of column efficiency when extra-column contribution is not taken into account is provided in the last chapter. When scaling up a column based on efficiency calculated for the system, column dimension could be over- or underestimated.

2. Theory

2.1. Column efficiency

Discussion on column efficiency began in a time when chromatography was still considered to be a succession of discontinuous equilibration steps with a step height and a specific time to achieve equilibration analogue to a classical distillation column [12]. Chromatography is now understood as a continuous process, but the idea of plates and plate height still holds and the number of plates (N) in a column serves as a dimensionless number for column efficiency, as explained in detail in an excellent review by Guiochon [12]. Column length (L) divided by N gives the height equivalent of the theoretical plate ($HETP$), which describes retention time distribution of the same molecular species within a sample moving through the column [3,12].

For interpretation of column performance, measured data of the system has to be corrected for influence of extra-column volume. N is calculated as ratio of squared mean retention time ($\bar{t}_R^2$) over peak variance (σ^2). Analyzing a chromatogram yields $\bar{t}^2_{R.System}$ and $\sigma^2{}_{System}$ which are used to calculate N_{System}. Assuming that individual contributions to peak broadening are independent of each other, column efficiency (N_{Column}) is calculated as shown in Eq. (1) [3,4]:

$$N_{Column} = \frac{\left(\bar{t}_{R.System} - \bar{t}_{R.ECC}\right)^2}{\sigma^2{}_{System} - \sigma^2{}_{ECC}} = \frac{\bar{t}^2_{R.Column}}{\sigma^2{}_{Column}} \quad (1)$$

where $\sigma^2{}_{ECC}$ is peak variance and $\bar{t}_{R.ECC}$ mean retention time of extra-column contribution. Through this subtraction of extra-column contribution from system data, influence of all construction parts of the extra-column volume, but also the inherent variance to the injection volume, are accounted for. As previously mentioned the reduction of N_{Column} to N_{System} is described as efficiency loss [8,14,15], meaning the loss of system efficiency due to extra-column contribution. In terms of retention times and variances, efficiency loss is described by Eq. (2):

$$N_{Column} > N_{System} \Rightarrow \frac{\bar{t}^2_{R.Column}}{\sigma^2{}_{Column}} > \frac{\bar{t}^2_{R.System}}{\sigma^2{}_{System}}$$

$$\Rightarrow \left(\frac{\bar{t}_{R.System} - \bar{t}_{R.ECC}}{\bar{t}_{R.System}}\right)^2 > \frac{\sigma^2{}_{System} - \sigma^2{}_{ECC}}{\sigma^2{}_{System}} \quad (2)$$

Rearranging Eq. (2) shows, if the ratio of variances is smaller than the ratio of squared mean retention times, N_{Column} is greater than N_{System}.

3. Materials and methods

3.1. Materials

3.1.1. Chemicals

All experiments used Milli-Q water as mobile phase. Tracers were D_2O, glucose, urea, and dextran T2000 (all four obtained from Sigma Aldrich, St. Louis, MO, USA). D_2O was used pure and in 1:5 dilution in Milli-Q water when 1 mL sample volume was applied. Dextran (3 g/L), urea, and glucose (both 5 g/L) were dissolved in Milli-Q water. The column was packed with Dowex Monosphere 99Ca/320 polystyrene resin (Supelco, Bellefonte, PA,