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Column-to-column packing variation of disposable pre-packed columns for protein chromatography

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ABSTRACT

In the biopharmaceutical industry, pre-packed columns are the standard for process development, but they must be qualified before use in experimental studies to confirm the required performance of the packed bed. Column qualification is commonly done by pulse response experiments and depends highly on the experimental testing conditions. Additionally, the peak analysis method, the variation in the 3D packing structure of the bed, and the measurement precision of the workstation influence the outcome of qualification runs. While a full body of literature on these factors is available for HPLC columns, no comparable studies exist for preparative columns for protein chromatography. We quantified the influence of these parameters for commercially available pre-packed and self-packed columns of disposable and non-disposable design. Pulse response experiments were performed on 105 preparative chromatography columns with volumes of 0.2–20 ml. The analyte acetone was studied at six different superficial velocities (30, 60, 100, 150, 250 and 500 cm/h). The column-to-column packing variation between disposable pre-packed columns of different diameter-length combinations varied by 10–15%, which was acceptable for the intended use. The column-to-column variation cannot be explained by the packing density, but is interpreted as a difference in particle arrangement in the column. Since it was possible to determine differences in the column-to-column performance, we concluded that the columns were well-packed. The measurement precision of the chromatography workstation was independent of the column volume and was in a range of ± 0.01 ml for the first peak moment and ± 0.007 ml^2 for the second moment. The measurement precision must be considered for small columns in the range of 2 ml or less. The efficiency of disposable pre-packed columns was equal or better than that of self-packed columns.

1. Introduction

Small scale columns of up to 20 ml are frequently used in biomanufacturing for process development, scale-down studies, exploration of the design space, and troubleshooting. For preparative separations, columns can either be bought as ready-to-use pre-packed columns or they are packed by the user himself. In the latter cases, only the bulk resin and the empty column hardware are bought from the manufacturer. Pre-packed preparative columns have become popular because the laborious column packing can be outsourced [1]. Pre-packed columns are available in non-disposable and disposable designs. Non-disposable columns are made of high quality materials such as glass walls and could be re-packed with a different medium by the customer, similar to self-packed columns. In comparison, disposable columns are made of cheaper materials such as polypropylene and cannot be re-packed. If the column lifetime is over, they are discared. Disposable columns must be simple and easy to manufacture in order to yield affordable columns. Self-packed chromatography columns are commonly tested before use to check the packing quality and to identify defects in order to ensure the reproducibility of runs. Frequently, pre-packed columns are used by customers with only limited additional qualification since the columns are assumed to have the same packing quality. However, only limited information is available to prove this assumption for preparative chromatography columns on the process development scale [2]. Differences in the column-to-column performance were investigated only for process-scale chromatography columns with diameters larger than 40 cm [3,4]. Ample of literature is also available for analytical [5–10], semi-preparative and preparative HPLC columns [11,12]. The column-to-column variation is more pronounced than the

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change of the column performance with time [11]. To our knowledge, a comparison of the packing quality of pre-packed columns to that of self-packed columns has not been performed.

The packed bed itself is highly heterogeneous in both the axial and radial directions [13–15]. The more homogeneous the packing, the lower the peak dispersion, measured either as height equivalent to a theoretical plate (H) or skewness [16]. It is known that the packing method [17] and the properties of the chromatography medium [2,18] influence the structure of the packed bed. Furthermore, the material [19,20] and the surface properties [21] of the column wall have an influence on column performance since they change the packing behavior. The packing density is an important factor to consider for evaluation of the column performance. It influences peak retention and width, since it is directly related to the extra particle porosity. Apart from the packed bed, the column performance also depends on the design of the column header [22] as well as on frits and filters [23].

Column performance is typically qualified by pulse response experiments of a small non-interacting solute. For small molecules, the main factor controlling column performance is hydrodynamic dispersion and not mass transfer. This allows evaluation of the column packing, which would be impossible with a biomacromolecule. It is assumed when the column is packed well enough to give good performance values for small molecules, it is also suitable for biomacromolecules. Pulse response experiments are highly dependent on the type of experimental testing set-up used and the method of peak analysis. The testing solute has an impact on the peak shape [24] and therefore must be kept constant for comparative studies. The amount of the injected sample affects the statistical moments of a peak [25] and peak analysis is also influenced by noise and baseline drift [26–29]. Proper baseline correction and setting of the integration intervals still allows the determination of higher moments with a good accuracy [30]. The two most commonly used peak analysis methods are direct numerical integration and peak fitting to a predefined function. The exponentially modified Gaussian (EMG) function [31,32] is the most popular function for peak fitting and provides robust results [33], especially for peaks with high experimental noise. The EMG was derived by convolution of a Gaussian peak with an exponential decay function. However, there is no physical reason, why a peak should follow the shape of an EMG [34]. Therefore, it fails to fit severe cases of tailing or fronting [34]. The peak parameters determined by EMG fitting can only be as good as the fit and hence do not reflect the real peak properties when the fit is bad. In comparison, direct numerical integration provides the most exact results [33], presuming the baseline drift is moderate and the data are smooth and without any noise.

In this study, we analyzed the performance of 0.2–20.0 ml pre-packed and self-packed preparative chromatography columns of different lengths and diameters that had been packed with different chromatography media in order to shed light on the scale-down of protein chromatography. The columns have been tested by injection of a non-interacting solute at different flow rates. The peak was evaluated by numerical integration and EMG fitting and the first and second peak moments and peak skewness were calculated and statistically evaluated with respect to column-to-column variation, measurement precision of the workstation, column types, and column dimensions.

2. Material and methods

2.1. Chemicals

Tris and sodium chloride were purchased from Merck Millipore and acetone was obtained from VWR chemicals. Silica particles (surface plain, size 1 μm, 50 mg/ml suspension in water) were purchased from Kisker Biotech GmbH & Co KG.

2.2. Columns

Pre-packed MiniChrom and ValiChrom columns from Repligen (previously Atoll) were used. MiniChrom columns are of disposable design while ValiChrom columns are non-disposable columns. The walls of the MiniChrom columns are made of polypropylene, while the ValiChrom columns are made of glass. The adapters of both column types are designed differently and have different volumes. The disposable columns are available at 2–3 pre-defined lengths. In contrast, the non-disposable columns are custom-made with any required length. All pre-packed columns have the same frit and filter at the top and at the bottom of the column (polypropylene/polyethylene fibre, weight 200 g/m^2, thickness 0.42 mm). The columns were packed with 4 different media: MabSelect SuRe (GE Healthcare, 85 μm particle diameter), Toyopearl Gigacap S–650 M (Tosoh, 75 μm particle diameter), Toyopearl SP–650 M (Tosoh, 65 μm particle diameter) and Toyopearl Phenyl–650 M (Tosoh, 65 μm particle diameter). MabSelect SuRe is a compressible Protein A medium with highly cross-linked agarose as backbone. Both, Toyopearl Gigacap S–650 M and Toyopearl SP–650 M media are strong cation exchange media with a methacrylate backbone. The Gigacap resin has an additional polymer linker between the backbone and the sulfopropyl functionalization. Toyopearl Phenyl–650 M has the same backbone as SP–650 M but is a hydrophobic interaction medium since it is functionalized with a phenyl ligand group. MiniChrom columns were supplied in complete sets of all available column sizes with the following diameter-length combinations (in mm): 5–10, 5–25, 5–50, 8–20, 8–50, 8–100, 11.3–50 and 11.3–100. Each of those column dimensions was delivered three times pre-packed with either MabSelect SuRe or Toyopearl Gigacap S–650 M. Three additional columns packed with MabSelect SuRe were available in the 11.3–50 dimension. Each column dimension was available once pre-packed with Toyopearl SP–650 M and Toyopearl Phenyl–650 M. ValiChrom columns packed with MabSelect SuRe and Toyopearl SP–650 M were delivered in the following diameter-length combinations (in mm): 5–100, 5–150, 5–200, 8–150, 8–200, 8–250, 11.3–100, 11.3–150 and 11.3–200. ValiChrom columns packed with Toyopearl Phenyl–650 M were available in the following diameter-length combinations (in mm): 5–100, 5–200, 8–150, 8–200, 11.3–150 and 11.3–200.

Additionally, we packed columns in our laboratory with MabSelect SuRe and Toyopearl Gigacap S–650 M using Tricorn 5 columns (GE Healthcare). They are designed as non-disposable columns with a diameter of 5 mm. Tricorn 5 filters (ethylene propylene diene/polyethylene, porosity 7 μm, thickness 1.35 mm) were used at the top and at the bottom of the columns without any frits. The columns were packed according to optimized packing protocols with bed heights in the range of 12–162 mm.

The described columns will hereafter be referred to as pre-packed disposable (MiniChrom), pre-packed non-disposable (ValiChrom), and self-packed (Tricorn) columns.

2.3. Workstation

An ÄKTA™ pure 25 M2 chromatography system (GE Healthcare) was used, which was controlled with Unicorn software 6.4. The extra column tubing between the pumps, valves, and detectors was used as provided by the manufacturer. The samples were injected via an injection loop. The injection valve has a total volume of 44 μl and the column valve of 110 μl. The detection cell of the UV detector has a volume of 15 μl. The tubing from the column valve to the column and back was varied based on the column type used. Tubings with an ID of 0.25 mm and a length of

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