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Trend analysis of performance parameters of pre-packed columns for protein chromatography over a time span of ten years

Theresa Scharl[a,b], Christian Jungreuthmayer[a], Astrid Dürauer[a,c], Susanne Schweiger[a], Tim Schröder[d], Alois Jungbauer[a,c,*]

[a] Austrian Centre of Industrial Biotechnology, Vienna, Austria
[b] Institute of Applied Statistics and Computing, University of Natural Resources and Life Sciences, Vienna, Austria
[c] Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
[d] Atoll, Weingarten, Germany

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ABSTRACT

Pre-packed small scale chromatography columns are increasingly used for process development, for determination of design space in bioprocess development, and for post-licence process verifications. The packing quality of 30,000 pre-packed columns delivered to customers over a period 10 years has been analyzed by advanced statistical tools. First, the data were extracted and checked for inconsistencies, and then were tabulated and made ready for statistical processing using the programming language Perl (https://www.perl.org/) and the statistical computing environment R (https://www.r-project.org/). Reduced HETP and asymmetry were plotted over time to obtain a trend of packing quality over 10 years. The obtained data were used as a visualized coefficient of variation analysis (VCVA), a process that has often been applied in other industries such as semiconductor manufacturing. A typical fluctuation of reduced HETP was seen. A Tsunami effect in manufacturing, the effect of propagation of manufacturing deviations leading to out-of-specification products, was not observed with these pre-packed columns. Principal component analysis (PCA) showed that all packing materials cluster. Our data analysis showed that the current commercially available chromatography media used for biopharmaceutical manufacturing can be reproducibly and uniformly packed in polymer-based chromatography columns, which are designed for ready-to-use purposes. Although the number of packed columns has quadrupled over one decade the packing quality has remained stable.

1. Introduction

Pre-packed columns or ready-to-use laboratory chromatography columns have been on the market for about one decade and have become very popular for process development and *a posteriori* evaluation of design space [1,2]. Such columns are used to reduce time for packing, which can be very tedious. Time savings is the major criterion for why pre-packed columns are widely applied in biopharmaceutical industry. It is assumed that the performance does not change over time and that consistent lots can be produced. These columns are often used to corroborate findings which have been made with automated systems either by parallel chromatography in robotics systems [3] or by adsorption measurements in microtiter plates [4–6]. For ready-to-use disposable columns, column construction must be simple and inexpensive. Adjustable pistons like adaptors are too expensive for this purpose. Therefore, a precise amount of chromatography medium must be packed into the column, which requires knowledge and skill in column packing. The performance of the packed columns is also checked by the manufacturer before sale. The customers assume that all ready-to-use columns display the same packing quality and can be applied without re-checking the packing quality. Interestingly, the quality can change to certain extent over time.

Manufacturing systems can be divided into 4 major models, transformation operations, operations of modification of structure, information operations, and transfer operations [7]. We can also assume that the same principles of manufacturing variations apply to column packing as used in other industry areas. Column packing can be considered as a transformation operation.

In principle, chromatography media can be categorized according to the backbone: (1) natural polymers such as agarose or

* Corresponding author at: Austrian Centre of Industrial Biotechnology, Vienna, Austria.
E-mail address: alois.jungbauer@boku.ac.at (A. Jungbauer).

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dextran; (2) synthetic polymers such as acrylamide or polymethacrylate; and (3) inorganic materials such as silica, hydroxy apatite, or controlled pore glass [8]. Many methods have been described for column packing taking into account the nature of the backbone. The influence of packing procedures on packing quality of small columns is still not fully understood [9]. Semi-rigid chromatography material is easier to pack than rigid particles and soft material such as Sephadex G-25 or Biogel [10–12]. Slurry packing under flow (flow packing) is routinely used for packing of chromatography material based on soft natural and synthetic polymers. The column is packed under a higher flow rate and pressure than applied during separation. Rigid particles are often dry-packed, but this depends on the particle size. The beads are filled into the column and are then compressed, a process also known as axial compression [13–17]. Such axial compression can be also applied for slurry packing, but is not feasible for ready-to-use columns because a piston is not part of the construction. Vibration of the column during packing may help to improve packing density. This technique has been successfully practiced for many years [18–20].

The standard parameters for controlling the packing quality of columns on all scales are HETP (Height Equivalent to a Theoretical Plate) and asymmetry [21,22]. HETP is a parameter which is independent of column length but depends on size of the particle, pore size, pore size distribution, particle porosity, velocity and the solute which is used for the experiment. When material dedicated for protein solution is tested with a small molecule tracer then the pore, pore size, pore size distribution and particle porosity is of less concern, because the molecules have a very high effective diffusivity. Asymmetry is another very good parameter to quantify the packing quality because it indicates if the packing density close to the column wall is lower or higher than in the center of the column. It is also a measure of the exponential wash out caused by extra column volume [23]. The experimental conditions to measure HETP and asymmetry must be standardized with respect to applied solute and velocity. It is also well known that the method of peak fitting may influence the outcome, e.g., graphical peak integration, numerical integration or fitting of the peak by a model function and calculation of variance and retention time based on the model. In addition to the experimental conditions, the data evaluation must be highly standardized in order to compare packing qualities among different experiments [24,25].

In order to see trends in a material property, a simple trend analysis can be performed. A simple but very effective way is to plot the property over time to visualize changes. The variation of the data can be assessed by, e.g., principal component analysis, which may help to identify co-variances. We received the performance data of 30,000 packing experiments of ready-to-use columns. Different chromatography media designed for purification of proteins and other large biomolecules were packed under standardized conditions. For each medium, an optimal procedure was developed by the column packing company. The packing quality was tested by injection of an aliquot of acetone and the retention time and peak width were determined from the peak profiles. The same procedure was used over the entire production period of approximately ten years. The injection of a small tracer molecule was selected since due to the large pores, only the hydrodynamic dispersion was measured. This dispersion is only influenced by the packing quality and the extra-column dispersion including the dispersion by the header and adapter.

An enormous challenge of this study was the extraction of the huge quantity of experimental data obtained from column testing. A proprietary closed-source software (Eurochrom) had been used to originally test the columns and to store the corresponding results. Eurochrom stores the data in a binary format and does not provide an API (application programming interface) that enables accessing the data via another program. Consequently, the column data could only be retrieved via Eurochrom's GUI (graphical user interface). For a small number of samples, a manual export is possible. However, the manual data export of 30,000 test runs which had been stored in 550 Eurochrom databases spread over 535,000 binary files was a very time consuming and error-prone process. Therefore, a computer software program was written in the programming language Perl with the module Win32:GuiTest which emulated mouse events and key strokes of a human computer user and utilized Eurochrom's GUI to automatically retrieve the column test data that had been generated over a period of ten years. After extraction, the data were read into the statistical computing environment R (https://www.r-project.org/) where the data was further processed, summarized, and visualized. Principal component analysis [26] was used to find the directions of the largest variations of the data, to visualize present structure in the data, and to detect outliers. This paper provides a useful and practical example of how preparative chromatography with ready-to-use columns can be standardized.

2. Materials and methods

2.1. Chromatography workstation and column packing

A chromatography workstation from Knauer, Berlin, Germany was used. The workstation was controlled by the Eurochrom software, which also handled data storage and peak analysis. Columns were packed by slurry packing under vibration.

2.2. Determination of HETP

HETP was measured by injection 50 μl of acetone or sodium nitrate and the UV 218 nm response was recorded. The chromatographic workstation automatically determined the number of plates N. From retention time (t) and N ($N = 5.54^{*} (t/W_{0.5})^2$) determined from the peak width measured at half peak height ($W_{0.5}$), the peak width (σ) was calculated by

$$\sigma = \sqrt{\frac{t^2}{N}} \tag{1}$$

An effective plate number was used for the data evaluation

$$N_{eff} = \left(\frac{t - t_0}{\sigma}\right)^2 \tag{2}$$

where t is the retention time, t_0 the dead time and σ^2 the variance.

Height equivalent to one theoretical plate (*HETP*) is defined as

$$HETP = L\frac{\sigma^2}{\mu^2} \tag{3}$$

with σ^2 the variance, L the column length, and μ the first peak moment. Reduced HETP (h) is obtained by dividing HETP by the particle diameter (d_p).

$$h = \frac{HETP}{d_p} \tag{4}$$

Asymmetry (A_s) was calculated at 10% peak height [27] with

$$A_s = \frac{b}{a} \tag{5}$$

a the width of the front part of the peak divided at peak maximum and b the width of the rear part.

Reduced velocity was calculated as

$$v = \frac{u \cdot d_p}{D_0} \tag{6}$$