reported by Larson, et al. (Larson, T.M., et al., Biotechnol. Prog. 19 (2003) 485-492). Moscariello, J., et al., J. Chrom. A 908 (2001) 131-141 report the characterization of the performance of industrial-scale columns. The resolution and column efficiency in chromatography is reported by Vink, H., J. Chrom. 69 (1972) 237-242. Sarker, M. and Guiochon, G., J. Chrom. A 702 (1995) 27-44 report a study of the packing behavior of axial compression columns for preparative chromatography.

The use of an integrated form of the Gaussian distribution function allows for the description of the packed bed characteristics, whilst neglecting effects outside the packed bed itself, which potentially influence the evaluation (see e.g. PCT/EP2010/003813). The implementation of equipment characteristics in the evaluation of non-Gaussian distributions observed during the assessment of packed chromatographic beds has been described by Guiochon, G., et al. (FUNDAMENTALS OF PREPARATIVE AND NONLINEAR CHROMATOGRAPHY; Guiochon, G., et al. (eds), Elsevier Inc., San Diego (USA), 2nd edition (2006)).

Summary ~~of the Invention~~

With the method as reported herein a determination of the decrease in the separation efficacy and/or packing quality of a re-useable chromatography column packing can be determined without the need to use and inject a further tracer compound prior to the separation of the crude polypeptide solution for the determination of column material integrity or the need for historical data of this purification step.

The method reported herein allows for determination of packed matrix parameters separately and independently from contributions and effects of the equipment involved. This allows for the discrimination and/or allocation of separate physical effects and for a comprehensive characterization of the chromatography equipment used independent of the scale, such as in analytical scale and in industrial scale.

The present disclosure provides~~first aspect as reported herein is~~ a method for determining whether a re-useable chromatography column packing, which is used at least for the second time in a purification step of a multi-step purification process of a polypeptide, has reduced separation efficacy, e.g. compared to the separation efficacy when it was used for the first time in the same purification step of the same multi-step purification process of the same polypeptide, comprising the following steps:

a) identifying and determining the experimental data of an inert change of at least one physicochemical parameter of a mobile phase passing through said re-useable chromatography column packing,

b) determining the parameters of a function of formula I by fitting the experimental data of the inert change of the physicochemical parameter of the at least second use determined in step a),

c) determining the difference between the experimental data of the inert change of the physicochemical parameter of the at least second use determined in step a) and the function of formula I with the parameters determined in step b),

d) calculating the difference between the maximum value and the minimum value of the difference determined in step c) and normalizing said difference,

e) determining reduced separation efficacy of said re-useable chromatography column packing when the absolute value of the difference calculated in step d) is more than 0.1,

wherein the function of formula I is

formula I

$$yI = -\frac{1}{2}A \cdot \exp\left(-\frac{x}{t_0}\right)\left(\exp\left(\frac{2x_c t_0 + w^2}{2t_0^2}\right)\left(erf\left(\frac{\left(\frac{x-x_c}{w} - \frac{w}{t_0}\right)}{\sqrt{2}}\right)+1\right) - \exp\left(\frac{x}{t_0}\right)\cdot erf\left(\frac{x-x_c}{\sqrt{2}\cdot w}\right)\right) + y_0$$

with parameters A and y_0 describing the respective signal changes, x_c the mean value, and w the standard deviation of the underlying Gaussian distribution function, respectively, and parameter t_0 describing the time constant of the underlying exponential decay function, and

wherein the erf-function used is defined as

$$erf(x) = \frac{2}{\sqrt{\pi}}\sum_{0}^{\infty}\frac{(-1)^n x^{2n+1}}{(2n+1)n!}.$$

In a first aspect, there is provided a method for determining whether a re-useable chromatography column packing, which is used at least for the second time in a purification step of a purification of a polypeptide, has reduced separation efficacy in said purification step of said purification of said polypeptide, characterized in that said method comprises the following steps:

a) identifying and determining the experimental data of an inert change of at least one physicochemical parameter of a mobile phase passing through said re-useable chromatography column packing of the at least second use,

b) determining the parameters of a function of formula I by fitting the experimental data of the inert change of the physicochemical parameter of the at least second use,

c) determining the difference between the experimental data of the inert change of the physicochemical parameter of the at least second use and the function of formula I with the parameters determined in step b),

d) calculating the difference between the maximum value and the minimum value of the difference determined in step c) and normalizing said difference,

e) determining reduced separation efficacy of said re-useable chromatography column packing when the absolute value of the difference calculated in step d) is more than 0.1,

wherein the function of formula I is

formula I

$$yI = -\frac{1}{2} A \cdot \exp\left(-\frac{x}{t_0}\right)\left(\exp\left(\frac{2x_c t_0 + w^2}{2t_0^2}\right)\left(erf\left(\frac{\left(\frac{x - x_c}{w} - \frac{w}{t_0}\right)}{\sqrt{2}}\right) + 1\right) - \exp\left(\frac{x}{t_0}\right) \cdot erf\left(\frac{x - x_c}{\sqrt{2} \cdot w}\right)\right) + y_0$$

with absolute signal values determined by the solvent employed (A and y_0), the mean value and the standard deviation of the underlying Gaussian distribution function (x_c, w), the time constant of the underlying exponential decay function (t_0), and wherein the erf-function used is defined as

$$erf(x) = \frac{2}{\sqrt{\pi}} \sum_{0}^{\infty} \frac{(-1)^n x^{2n+1}}{(2n+1)n!}.$$

The present disclosure also provides~~Another aspect as reported herein is~~ a method for the chromatographic purification of a polypeptide, in which at least one chromatography step with a re-useable chromatography column packing is comprised, comprising the following steps:

In a second aspect, there is provided a method for the chromatographic purification of a polypeptide, wherein at least one chromatography step using a re-useable chromatography column packing is contained, characterized in that said method comprises the following steps:

a) identifying and determining the experimental data of an inert change of at least one physicochemical parameter of a mobile phase passing through said re-useable chromatography column packing of the at least second use,

b) determining the parameters of a function of formula I by fitting the experimental data of the inert change of the physicochemical parameter of the at least second use,

c) determining the difference between the experimental data of the inert change of the physicochemical parameter of the at least second use and the function of formula I with the parameters determined in step b),

d) calculating the difference between the maximum value and the minimum value of the difference determined in step c) and normalizing said difference,

wherein the function of formula I is

formula I

$$yI = -\frac{1}{2} A \cdot \exp\left(-\frac{x}{t_0}\right)\left(\exp\left(\frac{2x_c t_0 + w^2}{2t_0^2}\right)\left(erf\left(\frac{\left(\frac{x - x_c}{w} - \frac{w}{t_0}\right)}{\sqrt{2}}\right) + 1\right) - \exp\left(\frac{x}{t_0}\right) \cdot erf\left(\frac{x - x_c}{\sqrt{2} \cdot w}\right)\right) + y_0$$

with absolute signal values determined by the solvent employed (A and y_0), the mean value and the standard deviation of the underlying Gaussian distribution function (x_c, w), the time constant of the underlying exponential decay function (t_0), and wherein the erf-function used is defined

$$erf(x) = \frac{2}{\sqrt{\pi}} \sum_{0}^{\infty} \frac{(-1)^n x^{2n+1}}{(2n+1)n!}$$

and further using the re-useable chromatography column packing when the absolute value of the difference calculated in step d) is 0.06 or less, or performing an additional characterization and/or assessment of the purified polypeptide when the absolute value of the

difference calculated in step d) is more than 0.06 but less than 0.2, or changing the re-useable chromatography column packing when the absolute value of the difference calculated in step d) is 0.2 or more.