US008357301B2

(12) **United States Patent**
Belousov et al.

(10) **Patent No.: US 8,357,301 B2**
(45) **Date of Patent: Jan. 22, 2013**

(54) **CHROMATOGRAPHY EQUIPMENT CHARACTERIZATION**

(75) Inventors: **Anton Belousov**, Penzberg (DE); **Thomas Dams**, Pullach (DE); **Benjamin Gerwat**, Weilheim i. OB (DE)

(73) Assignee: **Hoffmann-La Roche, Inc.**, Nutley, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/380,382**

(22) PCT Filed: **Jun. 22, 2010**

(86) PCT No.: **PCT/EP2010/003813**

§ 371 (c)(1),
(2), (4) Date: **Apr. 24, 2012**

(87) PCT Pub. No.: **WO2010/149367**

PCT Pub. Date: **Dec. 29, 2010**

(65) **Prior Publication Data**

US 2012/0202978 A1 Aug. 9, 2012

(30) **Foreign Application Priority Data**

Jun. 24, 2009 (EP) 09008247

(51) **Int. Cl.**
B01D 15/08 (2006.01)

(52) **U.S. Cl.** **210/656**; 210/198.2; 530/413; 702/30; 702/84

(58) **Field of Classification Search** 210/635, 210/656, 657, 659, 143, 198.2; 702/30, 81, 702/84, 104; 530/413
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,456,955 B1 * 9/2002 Andrews et al. 702/104

(Continued)

FOREIGN PATENT DOCUMENTS

WO 02/47009 6/2002

OTHER PUBLICATIONS

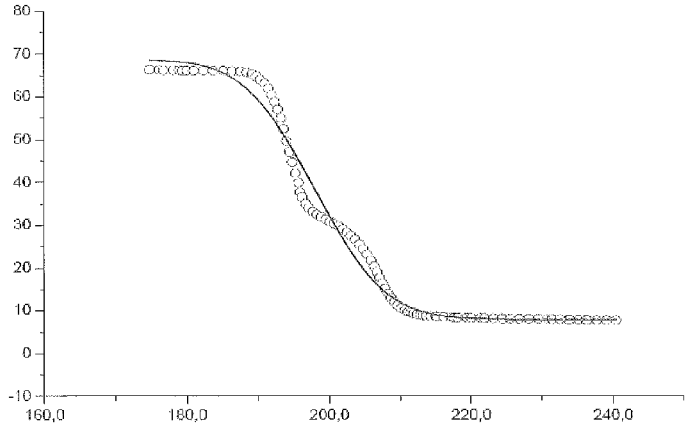
Sarker et al., "Study of the packaging behavior of axial compression columns for preparative chromatography" Journal of Chromatography A 702:27-44 (1995).

Larson et al., "Use of Process Data to Assess Chromatographic Performance in Production-Scale Protein Purification Columns" Biotechnol 19(2):485-492 (2003).

(Continued)

Primary Examiner — Ernest G. Therkorn
(74) *Attorney, Agent, or Firm* — Janet M. Martineau

(57) **ABSTRACT**

Herein is reported 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, 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, 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.

18 Claims, 8 Drawing Sheets