

Abstract

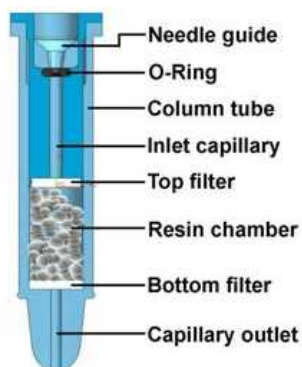
A new platform technology has been developed which enables 96 array format column chromatography. The design allows the user to select any chromatographic material which is packed with consideration to individual material requirements. Bed containment between two filter frits ensures high efficiency and peak symmetry similar to that of preparative and process separation columns, and distinguishes the system from the current filter based systems for simple on/off sample equilibration operation. Liquid flow in the columns (c.v. up to 0.6 mL) was driven with positive displacement fluid transfer systems, thus mimicking the situation in columns individually connected to a one channel stand-alone chromatography system. The combined robotic system (Atoll RoboColumn and Tecan Freedom EVO) allowed to perform automated high throughput small scale bio-chromatographic separations of protein samples by running up to eight individual columns simultaneously. These applications were successfully implemented for parameter elucidation and optimization in process development of therapeutic protein production, in-process monitoring of fermentation broth for mAb-production and sample preparation for mass spectrometry analysis in antibody screening.

Experimental Setup

A sealing inlet port for connection of small chromatography columns to fixed tips of a liquid delivery system was constructed, utilizing an appropriately sized Viton o-ring, located at the top of the column (Fig. 2). The inlet port of the RoboColumn column was checked for its ability to allow thousands of repeated insertions and removals of the fixed stainless steel tip, without becoming leaky. Utilizing injection moulding technique, a commercial version was developed (Fig. 2) with fixed bed height, made from polypropylene. For each experiment eight of these columns were packed individually with the desired chromatography material. The packed RoboColumns were mounted in eight-rows on standard 96-well compatible base plates which are accepted by a dedicated plate holder in a modified robotic workstation (Fig. 1 and 3). Chromatographic separations were carried out by applying an individually controlled flow of solutes (buffers, samples) to eight columns simultaneously in subsequent delivery steps by the eight channel liquid handler of an appropriately modified TECAN Freedom Evo workstation. Fractions were collected into microplates and automatically evaluated in a system-integrated microplate reader.



FIGURE 1
Te-Chrom plate holder with MediaScout® RoboColumn array.



Patent Pending PCT/DE 2006/000708

FIGURE 2
Schematic drawing of a small volume Mediascout® RoboColumn.
Column dimensions: ID 5 mm
Bed height 2.5 - 30 mm
Column volume: 50 - 600 µL
Packing media: any desired



FIGURE 3
Liquid handling workstation Tecan Freedom Evo. Modified for use with MediaScout® RoboColumns.

2D In-Process Bioreactor Monitoring

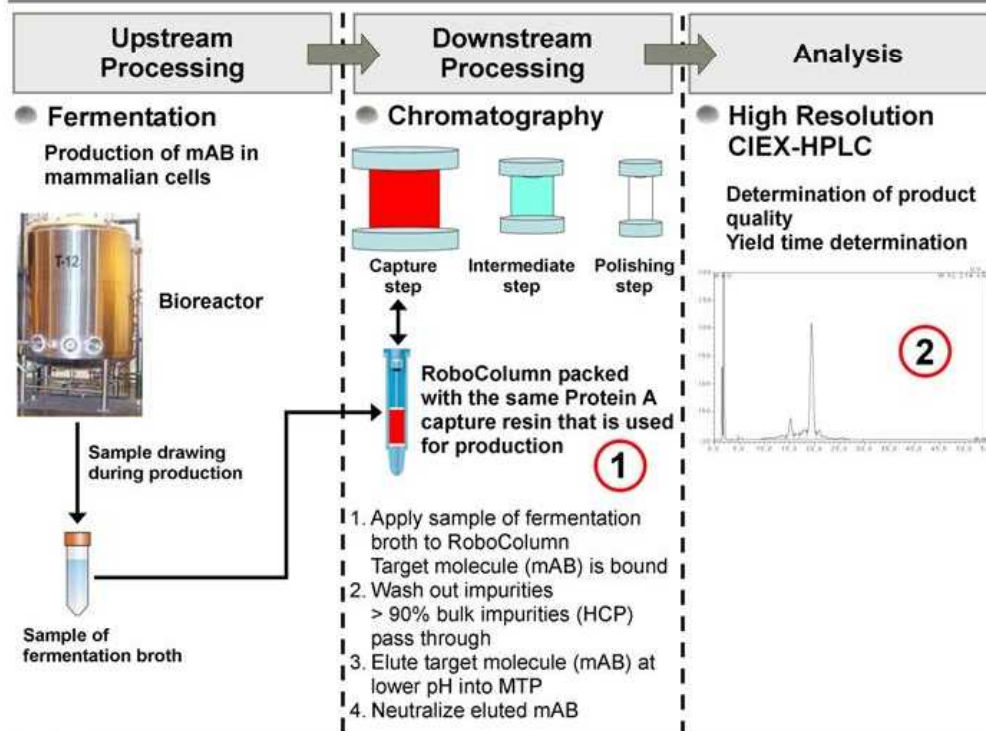


FIGURE 4
2D In-Process Bioreactor Monitoring. Process flow.

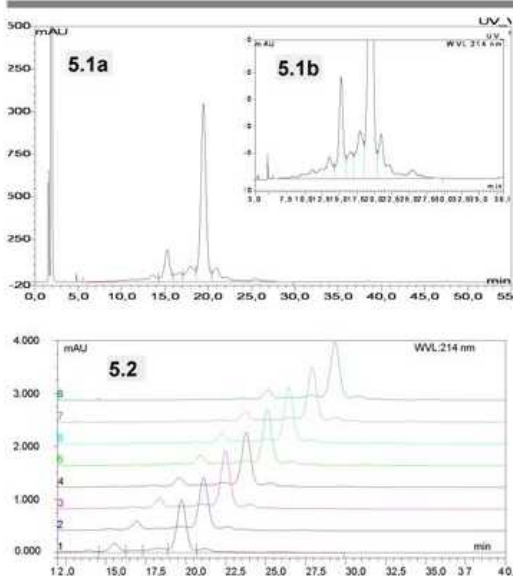


FIGURE 5
Among several rapidly implemented industrial applications with RoboColumns, the outcome of a rapid in-process monitoring of monoclonal antibodies from production scale fermentation broth is shown in Fig. 4. For this purpose samples from fermentation were loaded to an eight-row of RoboColumns, packed with affinity media.

Column: RoboColumn5-5 (CV 0.1 ml)
Media: ProSep®-vA High Capacity

After a short rinsing step the bound antibodies were rapidly eluted by an acidic buffer, immediately neutralized in the receiver plate by the liquid handler and submitted to high performance cation exchange chromatography (POROS® 20HS). The time consuming sample preparation step (Capture step) for this analysis could thus not only be automated, but also be accelerated by nearly one order of magnitude.

Sample Preparation for MS

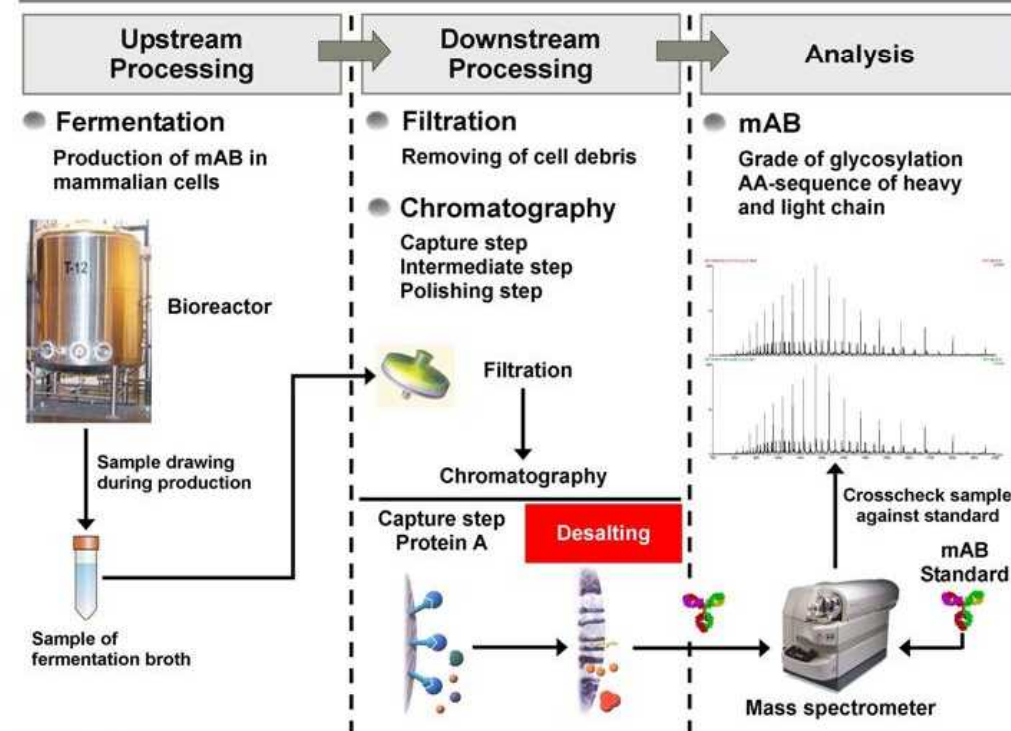


FIGURE 6
Sample preparation for mass spectrometry. Process flow.

FIGURE 7.1
MS after non continuous chromatography. Liquid flow induced by gravity.
Column: illustra NAP™-5 (GE Healthcare)
Dimensions: 9.5 x 28.7 mm (CV 2.0 ml)
Packing media: Sephadex™ G-25 DNA grade
Flow rate: n.a. (gravity)
Process time: 50 min/8 samples

FIGURE 7
Mass spectra of reduced mAb after fractional desalting using different operational modes show a perfect match.
Sample: 90 µg reduced mAb in 60 µl 20% C₂H₅N/1% HCOOH
c(mAb) = 1.50 µg/µl

FIGURE 7.2
MS after true continuous, parallelchromatography. Liquid flow driven by positive liquid displacement and controlled through dispensing speed of the robotic system.
Column: MediaScout® RoboColumn
Dimensions: 5 x 30 mm (CV 0.6 ml)
Packing media: Sephadex™ G-25 Superfine
Flow rate: 0.5 ml/min (150 cm/h)
Process time: 5 min/8 samples

Conclusions

- Atoll's 96 MediaScout® RoboColumn array was successfully adapted on TECAN's liquid handling workstation Freedom Evo® for chromatographic applications.
- The transfer from a common one channel stand-alone chromatography system into a fully automated, parallel chromatography system was successfully completed.
- Small scale automated HTP separations by bio-chromatography were successfully applied in screening of cell culture supernatants for recombinant monoclonal antibodies, both in research and development, as well as in full scale production.
- Time consuming manually desalting of protein samples (mAb) using common gravity induced columns, was effectively replaced by automated HTS-chromatography using robotically operated MediaScout® RoboColumns. Process time per sample was reduced about 10 times.
- Costs per experiment could be significantly reduced due to saving process time, API, process-relevant products and solvents.