Science Together

Applications of Simulated Moving Bed chromatography for continuous purification of small and large molecules

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SUMMARY

SMB chromatography is a HPLC technique for the separation and purification of binary mixtures with high productivity and purity. The KNAUER AZURA® SMB systems allow their usage in various areas such as the pharmaceutical or food industry. SMB is ideal to be used in the production of fine chemicals (small molecules) i.e. xylitol as well as in bioengineering for production of proteins (large molecules) i.e. antibodies. The two presented separation modes reveal the flexibility of the modular concept of the AZURA SMB systems for the purification of small and large molecules.

BENEFITS OF SMB CHROMATOGRAPHY

Simulated Moving Bed chromatography is a continuous chromatography technique that enables the separation of binary or pseudo-binary mixtures into pure substances or fractions. This process continuously separates and extracts two fractions thus leading to higher yields of purified substances while consuming less eluent and packing material compared to traditional batch chromatography. In classical one-column chromatographic separations only a small part of the column contributes in the separation, while the major part remains unused. This results in a significantly lower productivity compared to a SMB process where the whole column bed is efficiently used.

The modular concept of the KNAUER AZURA SMB systems allows a flexible adaption to different separation tasks (**Fig. 1**). The classical eight column configuration with two columns per zone can be easily adapted for optimization of the process by changing the numbers of columns per zone. Also, it can be switched between open and closed loop mode.

APPLICATION OF SMB FOR PURIFICATION OF XYLITOL

The application of a classical SMB eight column process in an open loop mode (**Fig. 2**) is shown by the purification of xylitol from fermentation mash of a fed-batch culture. Xylitol is the sugar alcohol of xylose which is mainly used in food industry as glucose replacement e.g. in chewing gums. It is classically produced by chemical conversion of xylose from birch to xylitol. Here, a Candida yeast strain was used to convert xylose from a hemicellulose hydrolysate to xylitol. HPLC analysis of the fermentation mash revealed that the xylose to xylitol conversion was successful and that a SMB process is feasible. Polymer based Eurokat Ca columns were selected for the separation process as they are customized for sugar separation and water is used as eluent. After determination of method parameters, a SMB process was designed and run. HPLC analysis of the two outlets, extract and raffinate revealed that xylitol was successfully separated and purified (**Fig. 3** & **4**). In one hour 1.8 g of xylitol was purified with 99 % purity and 100 % recovery (results not presented here, for further information see KNAUER Application VFD0157).

Fig. 1 AZURA® SMB Lab system

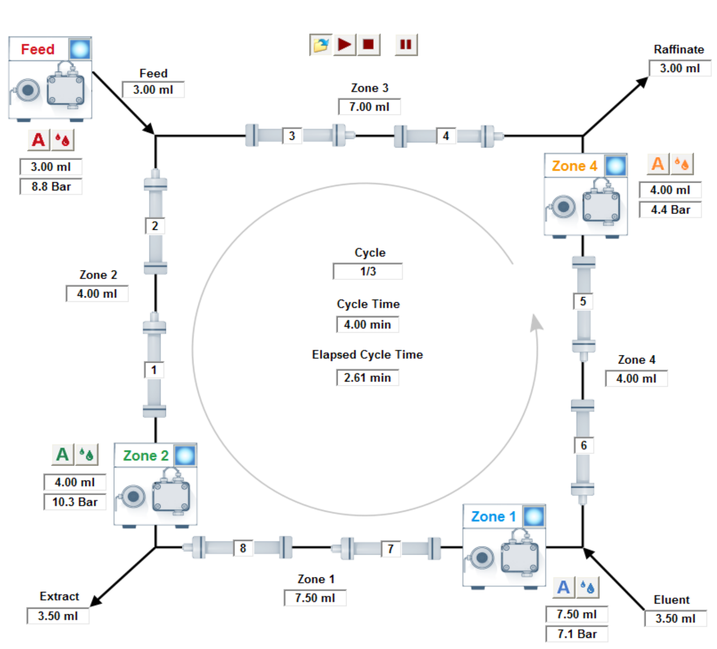


Fig. 2 Example scheme of the SMB process set-up with four pumps, out – and inlets, 8 columns, four zones, indication of flow rates, pressure, and cycle time; PurityChrom® MCC software

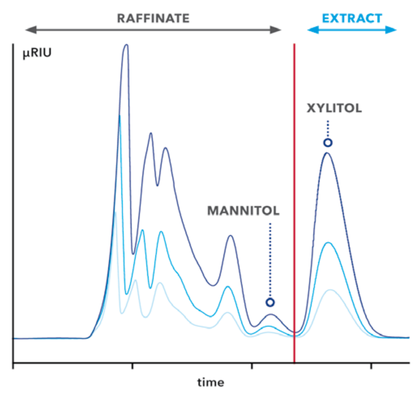


Fig.3 Semi-preparative chromatogram of fermentation mash; injection: light blue – 0.5 ml, blue – 1.0 ml, dark blue 2.0 ml

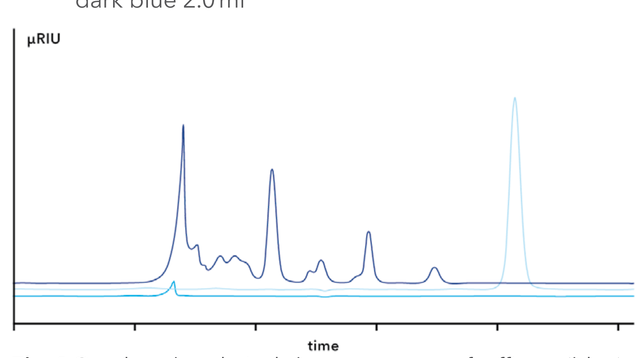
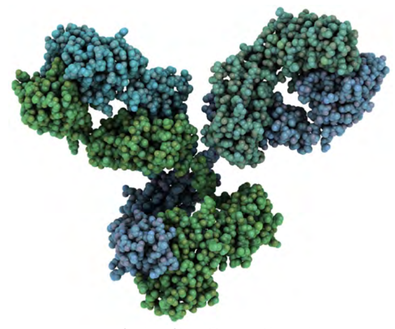


Fig.4 Overlayed analytical chromatograms of raffinate (blue); extract/xylitol (light blue); waste (blue)

APPLICATION OF SMB FOR PURIFICATION OF BIO MOLECULES

The same AZURA SMB system and the Multi-Column Capture Process (MCCP) can be used for the continuous purification of proteins such as antibodies in a capture mode. The flexible adaption of the number of columns to the different purification steps (binding, washing, eluting, and regeneration) results in a time saving, column cost and solvent reducing purification of target proteins. The software supports an easy switch from classical SMB to the MCCP process (**Fig. 5**). For the MCCP process, detectors can be additionally integrated into the system for monitoring and regulation of the process.



Immunoglobulin G antibody as an example for a large molecule

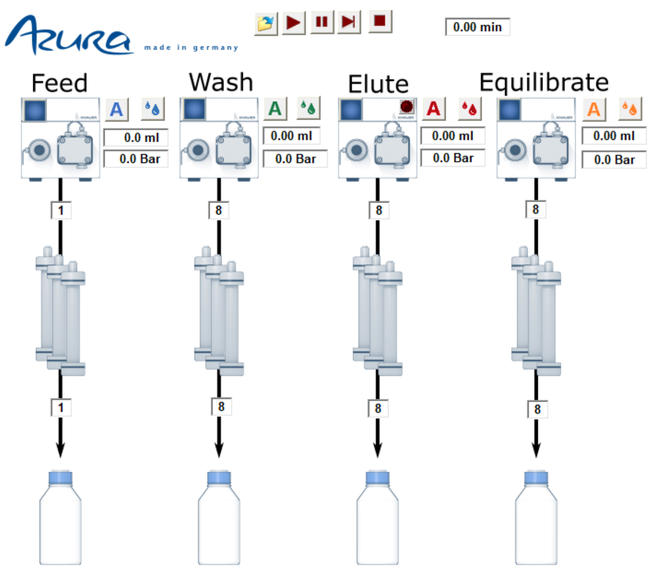


Fig. 5 Software user interface of MCCP process

Link to KNAUER Application VFD0157