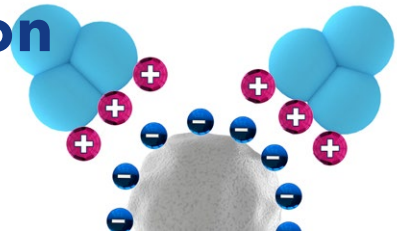
Science Together

KNAUER

Automated two step purification with cation exchange chromatography and desalting



Ulrike Krop, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; www.knauer.net.

SUMMARY

In many cases protein purification requires two or even more steps to achieve sufficient purity. The transition from one step to another can be very time consuming and requests manual interaction. These limitations illustrate the need for FPLC systems enabling automated two step purification. This application highlights the possibility of combining two subsequent chromatography protocols. This was achieved using the KNAUER Lab standard Multi Method FPLC system adapted for automatic two step purification. An exemplary separation of model proteins by cation exchange chromatography followed by desalting is shown. This approach can easily be adapted to various protein purification protocols.

INTRODUCTION

For many protein purifications several chromatographic steps are used to achieve pure protein. Depending on the desired yield and purity of the target protein different methods are combined. In many cases two steps for protein purification are used. For the two step purification two independent methods, each with their associated specific column, are used to realize the purification of the target molecule without manual interference. The general principle is that the protein sample is applied on the first column. During elution of the protein, the protein peak is detected triggering its collection in a storage loop or storage vessel. Subsequently, the target protein is automatically applied on the second column to further enhance the quality and/or purity. The fast and automated connection of two chromatographic purification steps into one method eliminates manual sample handling and minimizes time spent between steps. This automation strategy can be easily adapted to several purification tasks increasing your efficiency and optimizing the workflow. In this application note a KNAUER Lab standard Multi Method FPLC system for all bio chromatography methods was adapted for two step purification. Two model proteins cytochrome C and ribonuclease A were separated by cation exchange chromatography. Cytochrome C was automatically stored in a sample loop and applied onto a second column for buffer exchange with a desalting column.

Automated two step purification with cation exchange chromatography and desalting

Science Together

RESULTS

The first step of the separation was cation exchange chromatography. The proteins where chosen due to their positive charge in buffer at pH 6.8. Ribonuclease A has an isoelectric point of 9.6, while for cytochrome C a range from 10.37 to 10.80 for the isoelectric point is reported. With the starting conditions both proteins were binding to the cation exchange column. During gradient elution first ribonuclease A and later cytochrome C eluted from the column **(Fig. 1)**. A threshold signal **(Fig. 1**, red line) was used to detect cytochrome C triggering the intermediate storage in the sample loop. The cytochrome C was subsequently reinjected on the desalting column to exchange the buffer to the final buffer conditions **(Fig. 2)**. The eluted protein was fractioned.

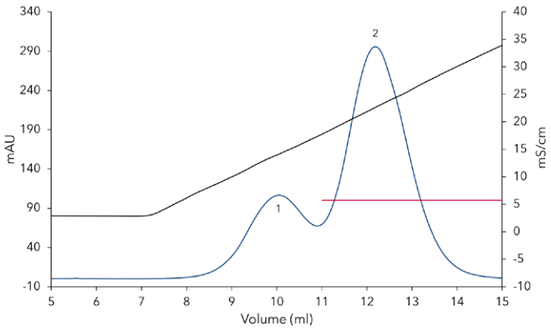


Fig. 1 Chromatogram of the cation exchange method. Blue line: UV 280 nm (blue), conductivity (black), threshold used for peak recognition (red). 1 - Ribonuclease A, 2 - Cytochrome C.

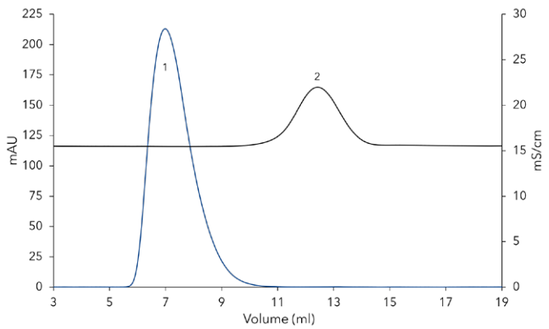


Fig. 2 Chromatogram of the desalting method. UV 280 nm (blue), conductivity (black). 1 - Cytochrome C, 2 - Salt peak.

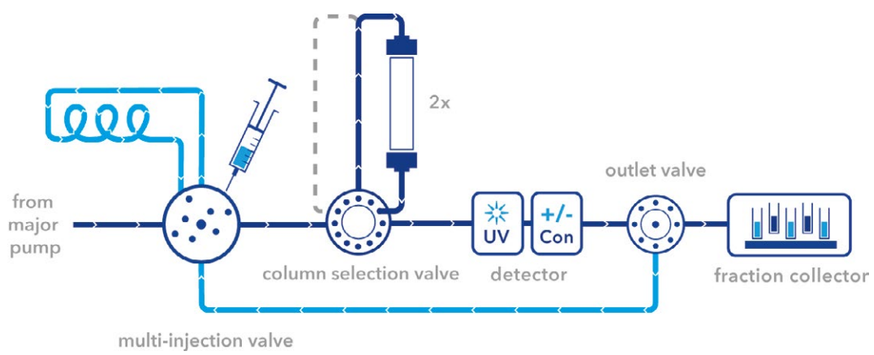


Fig. 3 Flow scheme of the lab standard KNAUER Multi Method FPLC system for all bio chromatography methods adapted for two step purification with the basic set up.

SAMPLE PREPARATIONS

Lyophilized cytochrome C from bovine heart (CAS# 9007-43-6) and lyophilized ribonuclease A from bovine pancreas (9001-99-4) was used. Both proteins were dissolved in buffer A to a final concentration of 0.5 mg/ml. In total, 2 ml of sample (1 mg of each protein) was injected for each purification. The sample was filtered before use (0.45 µm). A Lab standard KNAUER Multi Method FPLC system for all bio chromatography methods was adapted for two step purification. A column selection valve and an outlet valve were added to the system. This shows the basic set up for two step purification. The reinjection position of the outlet valve was connected to the sample pump inlet of the injection valve. The sample is injected via the sample loop of the injection valve. The first peak is collected in the sample loop of the injection valve as well and reinjected onto the second column.

CONCLUSION

The model protein cytochrome C was successfully purified from ribonuclease A by an automated combination of a cation exchange chromatography and a desalting method on the Lab standard KNAUER Multi Method FPLC system in the basic set up for two step purification. No manual interaction was necessary. This method setup could easily be adapted to other purification protocols for the separation and purification of biomolecules highlighting.

RELATED KNAUER APPLICATIONS

VBS0070 – Ion exchange chromatography with AZURA® Bio purification system
VBS0072 – Separation of proteins with cation exchange chromatographyon Sepapure SP and CM
VBS0075 – Group separation with Sepapure Desalting on AZURA® Bio purification system

MATERIALS AND METHODS

Tab. 1 Method

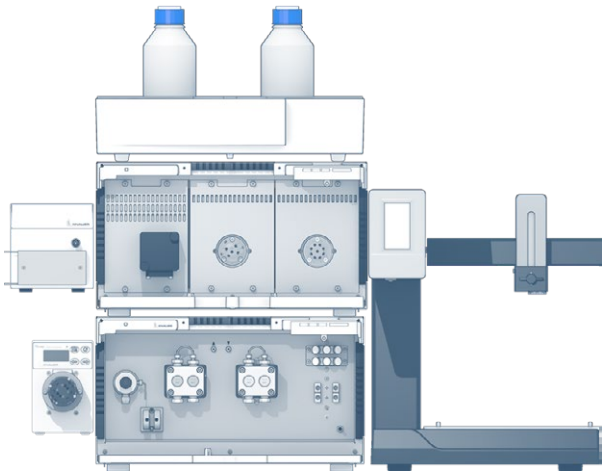
Column temperature	RT
Sample loop volume	2 ml
UV Detection	280 nm
Data rate	2 Hz
Buffer A	25 mM sodium phosphate buffer pH 6.8
Buffer B	25 mM sodium phosphate buffer pH 6.8 + 1M NaCl
Buffer C	PBS 0.01 M phosphate buffered saline (NaCl 0.138 M; KCl - 0.0027 M); pH 7.4

Tab. 2 Cation exchange method

Column	Sepapure CM FF6 1 ml
Flow rate	1 ml/min
Gradient	5 ml step 100% A 10 ml gradient 100% A to 60% A / 40% B
Peak recognition	10-15 ml Threshold: UV 280 nm 100 mAU

Tab. 3 Desalting

Column	Sepapure desalting 5 ml
Flow rate	2.5 ml/min
Gradient	1.5 CV isocratic buffer C



Tab. 4 Wash and equilibration of cation exchange method

Column	Sepapure CM FF6 1 ml
Flow rate	1 ml/min
Gradient	5 ml step 100% B 10 ml step 100% A

Tab. 5 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L, LPG Metal-free, low pressure gradient FPLC pump with 10 ml pump head, ceramic	APH64EB
Docking station	AZURA Assistent ASM 2.2L	AY0001
UV detector	Left module: UV detector UVD 2.1S	EDA03XA
Valve drive	Middle module: Valve drive VU 4.1	EWA04
Valve drive	Right module: Valve drive VU 4.1	EWA04
UV flow cell	Semi-preparative biocompatible 3 mm UV flow cell	A4045
Injection valve	AZURA V 4.1 Valve Multi-injection valve, biocompatible, 1/16"	AVN94CE
Outlet valve	AZURA V 4.1 Valve Biocompatible multiposition valve, 8 Port	AVS34CE
Column selection valve	VICI column selection valve Biocompatible column selection valve, for 5 columns, 1 bypass, reverse flow, 12 ports, 1/16", 50 bar	AVZ52CE
Valve drive	AZURA Valve unifier VU 4.1 Smart valve drive with RFID technology for valves V 4.1	AWA01XA
Conductivity monitor	AZURA CM 2.1S with flow cell up to 100 ml/min Conductivity monitor with flow cell for up to 100 ml/min and optional pH measurement	ADG30GD
Fraction collector	Fraction collector Foxy R1	A59100
Software	PurityChrom® Basic	A2650
Column 1	Sepapure CM FF6 1 ml, weak cation exchange, prepacked 1 ml, 100 µm, 3 bar	010X15QSPZ
Column 2	Sepapure desalting 5 ml, prepacked 5 ml, 20 - 50 µm, 3 bar	020X460SPZ