Science Together

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Comparison of ion exchange columns

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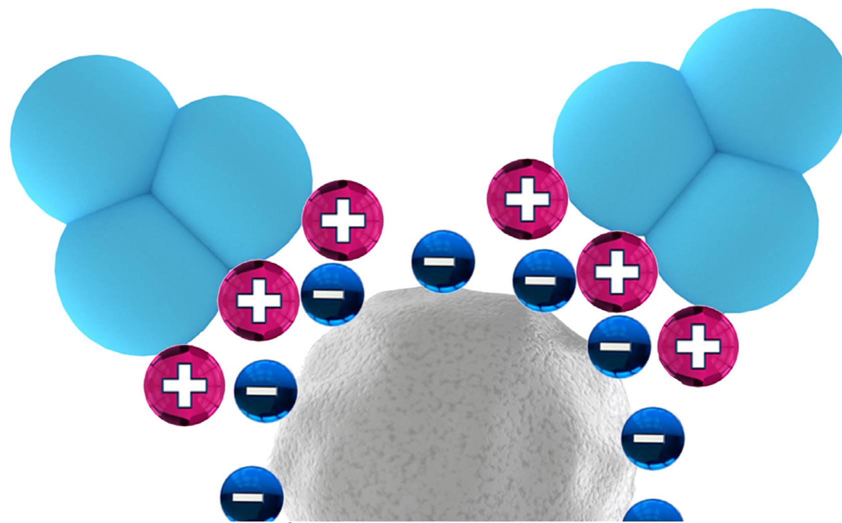
SUMMARY

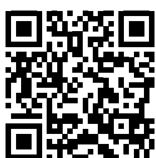
Ion exchangers are used in a variety of protein purification protocols. This application compares equivalent columns, a weak and a strong anion exchanger as well as a weak and a strong cation exchanger from two different vendors. The columns were comparable in all assessed cases.

INTRODUCTION

Ion exchange chromatography is a powerful technique to separate proteins and is therefore used in numerous purification protocols. The column resins are modified by covalently bound functional groups. The choice of column modification influences the selectivity of the column. According to the charge of the protein cation or anion exchange chromatography is the best method. In this application equivalent columns with different modifications from two vendors were compared.



Principle of cation exchange chromatography



Additional Information

Comparison of ion exchange columns

RESULTS

Cytochrome C (pI 10.3), Lysozyme (pI 11.35) and Ribonuclease A (pI 9.6) are proteins with relatively high pI values, which make them ideal candidates for cation exchange chromatography **(Fig 1 & 2)** while for anion exchange chromatography Conalbumin (pI 6.8), α-Lactalbumin (pI 5.8) and soy bean Trypsin inhibitor Cytochrome C (pI 4.5) were used **(Fig 3 & 4)**. All sample mixes bound under low salt conditions to the resin and eluted under increasing salt concentrations. Identical protein mixes and method parameters were used for the comparison of the two vendors of weak and strong anion and cation exchangers. The peaks for the protein separation are comparable in all evaluated cases.

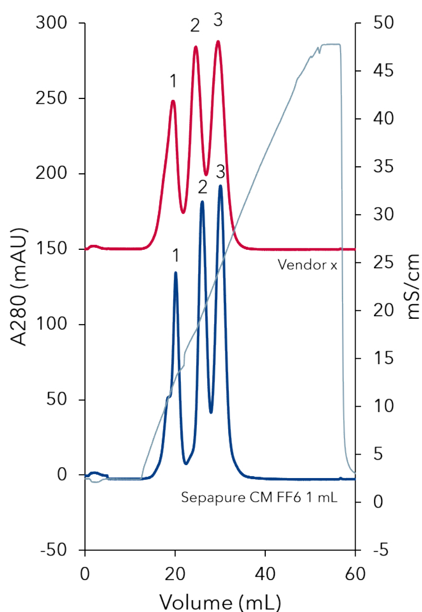


Fig. 1 Chromatograms of the separation of Ribonuclease A (1), Cytochrome C (2), and Lysozyme (3) with weak cation exchange chromatography columns, blue line: Sepapure CM FF6 1 mL, red line: comparable column from vendor x, grey line: conductivity signal

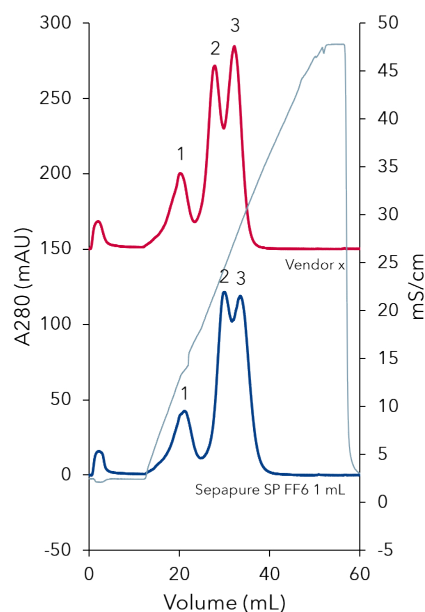


Fig. 2 Chromatograms of the separation of Ribonuclease A (1), Cytochrome C (2), and Lysozyme (3) with strong cation exchange chromatography columns, blue line: Sepapure SP FF6 1 mL, red line: comparable column from vendor x , grey line: conductivity signal

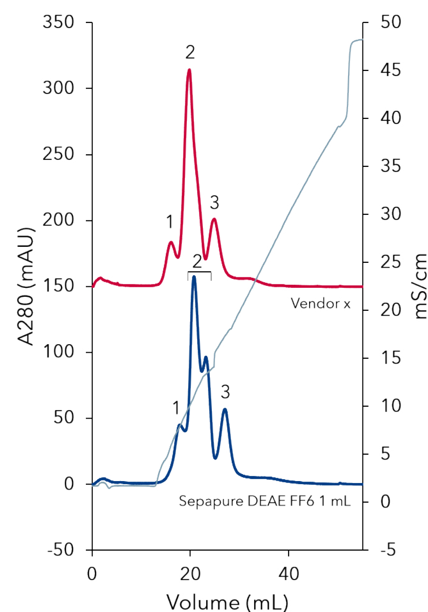


Fig. 3 Chromatograms of the separation of Conalbumin (1), α-Lactoalbumin (2), and soy bean Trypsin inhibitor (3) with weak anion exchange chromatography columns, blue line: Sepapure DEAE FF6 1 mL, red line: comparable column from vendor x, grey line: conductivity signal

MATERIALS AND METHODS

In this application, an AZURA Bio purification system consisting of AZURA P 6.1L LPG metal-free pump with 10 mL pump head; AZURA ASM 2.1L assistant module with an injection valve and a single wavelength UV detector UVD 2.1S; AZURA CM 2.1S conductivity monitor and Foxy R1 fraction collector was used. For cation exchangers a mix of Cytochrome C (0.4 mg/mL), Lysozyme (0.4 mg/mL), and Ribonuclease A (1 mg/mL) was used. For anion exchangers a mix of Conalbumin (0.2 mg/mL), α-Lactalbumin (0.4 mg/mL), and soy bean Trypsin inhibitor (0.6 mg/mL) was used. Prior to the run the 1 mL columns (Sepapure SP, CM, Q, DEAE and the equivalent columns from vendor x) were equilibrated in buffer A (for cation exchangers: 20 mM Sodium phosphate buffer pH 6.8; for anion exchangers: 20 mM Tris/HCl pH 7.4). 2 mL of the sample was injected with a flowrate of 1 mL/min. The columns were washed with 10 column volume (CV) of buffer A to remove all unbound protein. The proteins were eluted with a linear gradient over 40 CV up to 50 % buffer B (20 mM Sodium phosphate buffer pH 6.8, 1 M NaCl) for the cation exchangers or up to 40 % buffer B (20 mM Tris/HCl pH 7.4, 1 M NaCl) for the anion exchangers. The proteins were detected at 280 nm and conductivity signal was recorded to monitor the salt gradient.

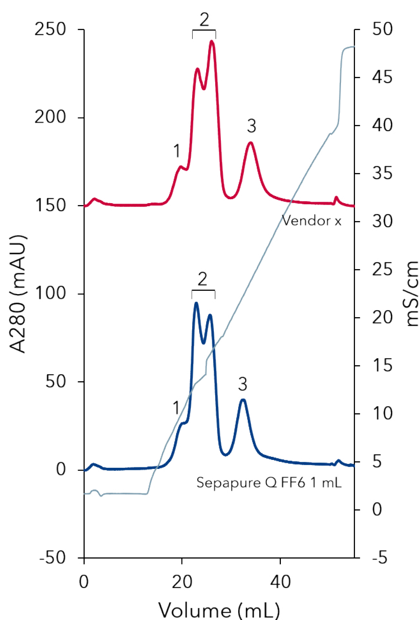
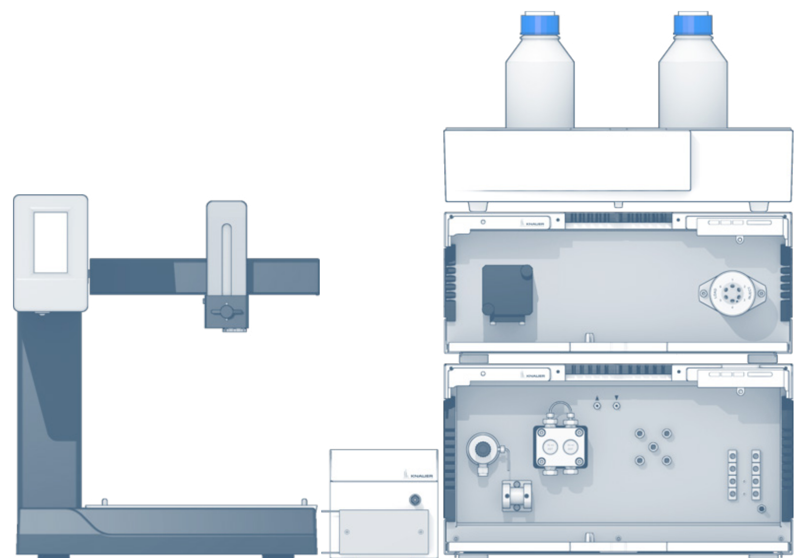


Fig. 4 Chromatograms of the separation of Conalbumin (1), α-Lactoalbumin (2) and soy bean Trypsin inhibitor (3) with strong anion exchange chromatography columns, blue line: Sepapure Q FF6 1 mL, red line: comparable column from vendor x, grey line: conductivity signal

CONCLUSION

Four different ion exchange columns types (SP, CM, Q, DEAE) from two vendors were compared. The equivalent columns were evaluated under identical conditions. The chromatograms of the protein separation are comparable in all assessed cases. The alternative columns can be considered as a replacement.



ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters for cation exchange chromatography runs

Eluent A	20 mM Sodium phosphate buffer pH 6.8		
Eluent B	20 mM Sodium phosphate buffer pH 6.8 + 1 M NaCl		
Gradient	Volume [mL]	% A	% B
	10 step	100	0
	40 gradient	50	50
	5 step	50	50
	10 step	100	0
Flow rate	1 mL/min (2 mL/min from 50 mL)	System pressure	>3 bar
Run temperature	RT	Run time	57.5 min
Injection volume	2 mL	Injection mode	Automatic injection valve
Detection UV	280 nm	Data rate	2 Hz

Tab. A2 Method parameters for anion exchange chromatography runs

Eluent A	20 mM Tris/HCl pH 7.4		
Eluent B	20 mM Tris/HCl pH 7.4 + 1 M NaCl		
Gradient	Volume [mL]	% A	% B
	10 step	100	0
	40 gradient	60	40
	5 step	50	50
	10 step	100	0
Flow rate	1 mL/min	System pressure	>3 bar
Run temperature	RT	Run time	~60 min
Injection volume	2 mL	Injection mode	Automatic injection valve
Detection UV	280 nm	Data rate	2 Hz

Tab. A3 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L, LPG 10 mL PEEK PK	APH69EB
Assistant	AZURA ASM 2.1L Right: UVD 2.1S Middle: - Left: V2.1S 6 Port/6 Position PEEK 1/16"	AYCALXEC
Flow cell	3 mm semiprep, 2 µL, biocompatibel	A4045
Conductivity monitor	AZURA CM 2.1S	ADG30
Flow cell	Preparative up to 100 mL/min	A4157
Column	Sepapure Q FF6 1mL Sepapure DEAE FF6 1mL Sepapure SP FF6 1mL Sepapure CM FF6 1mL vendor x Q FF 1mL vendor x DEAE FF 1mL vendor x SP FF 1mL vendor x CM FF 1mL	010X15HSPZ 010X15ISPZ 010X15RSPZ 010X15QSPZ
Fraction collector	Foxy R1	A2650
Software	Purity Chrom	A59100

RELATED KNAUER APPLICATIONS

VBS0070 – Ion Exchange Chromatography with AZURA® Bio purification system

VBS0071 – Comparison of two column sets for antibody purification in an automated two step purification process

VBS0072 – Separation of proteins with cation exchange chromatography on Sepapure SP and CM

VBS0073 – Separation of proteins with anion exchange chromatography on Sepapure Q and DEAE

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