Science Together

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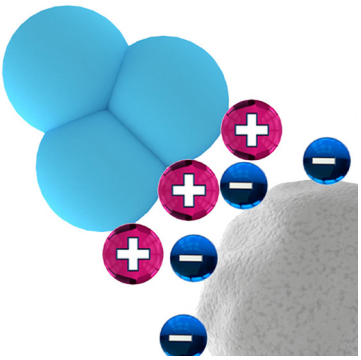
Ion Exchange Chromatography with AZURA® Bio purification system



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SUMMARY

Ion exchange chromatography is a popular technique for protein separation and purification. This application describes the separation of three model proteins by a salt gradient with the AZURA® Bio purification system.

INTRODUCTION

Ion exchange chromatography separates molecules based on the overall charge of the protein. The proteins of interest have a charge opposite to that of the resin. In the case of cation exchange chromatography, proteins have an overall negative charge while binding to a cationic column **(Fig 1)**. The initial binding takes place under low ionic strength conditions. Elution is achieved by a salt gradient. By increasing the salt concentration proteins with a weak negative charge elute first, while at higher salt concentrations proteins with a strong negative charge elute later. Ion exchange chromatography is frequently used for protein purification. A precise gradient formation is here one important parameter for a successful separation of proteins. AZURA Bio purification system supports all gradient methods including ion exchange chromatography.

Additional Information

Ion Exchange Chromatography with AZURA® Bio purification system

RESULTS

Because of their isoelextric point (pI) the three proteins: α-Chymotrypsinogen A (pI 8.97), Cytochrome C (pI range from 10.0 – 10.5), and Lysozyme (pI 11.35) are well suited for the separtion by cation exchange chromatography. At pH 6.1 all three model proteins have an overall negative charge and bind to the resin under low salt conditions. Remaining impurities and potentially unbound proteins are washed from the column during the wash step. By slowly increasing the salt gradient, first α-Chymotrypsinogen A **(Fig 2** blue signal, Peak 1**)** eluted from the column followed by Cytochrome C (Peak 2) and Lysozyme (Peak 3). The salt gradient was monitored by the conductivity monitor **(Fig 2** red signal**)**.

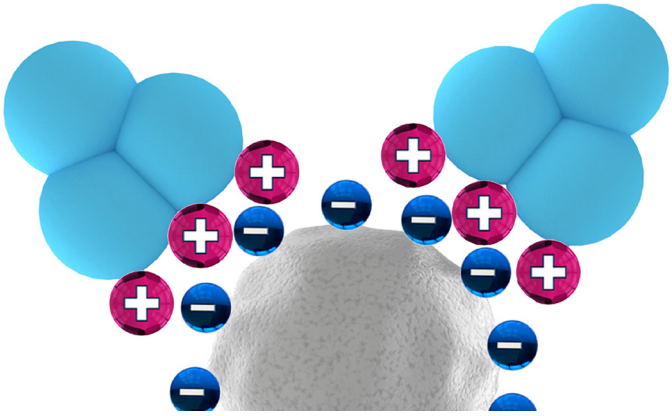


Fig. 1 Principle of cation exchange separation Proteins with different negative charges bind to the cation exchange resin. By increasing the salt concentration proteins with a weak negative charge elute first, while at higher salt concentrations proteins with a strong negative charge elute last.

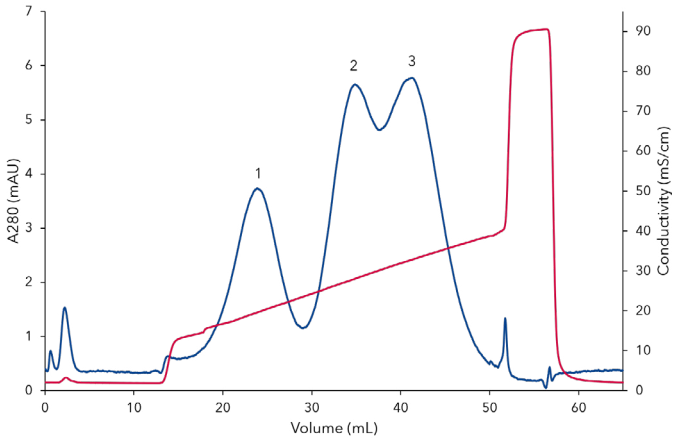
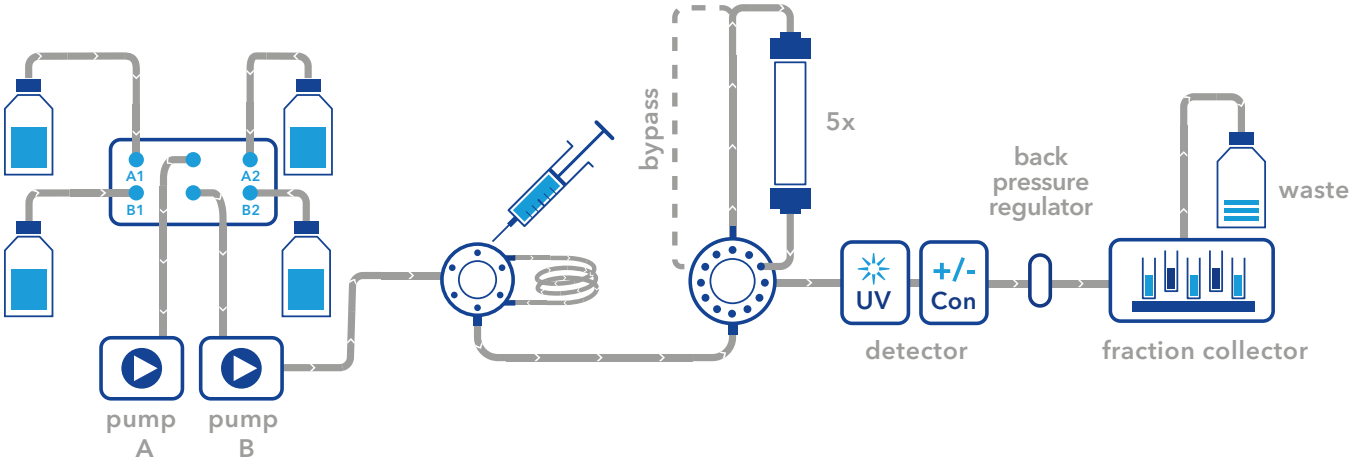


Fig. 2 Chromatogram of the separation of three model proteins with cation exchange chromatography, blue line – UV 280 nm signal, red line – conductivity signal, 1) peak containing α-Chymotrypsinogen A, 2) peak containing Cytochrome C, 3) peak containing Lysozyme.



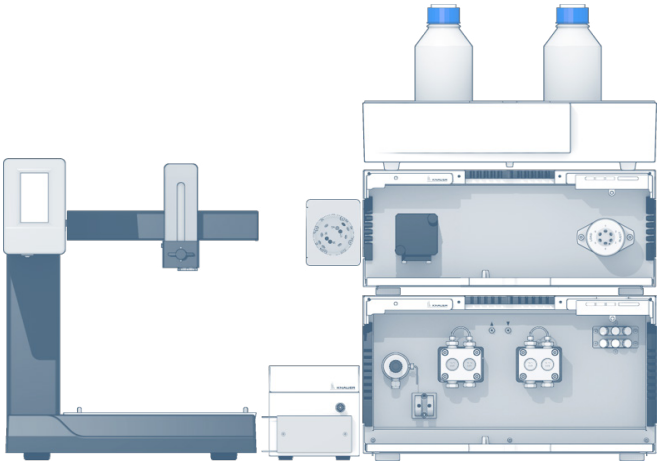
BUFFER SELECTION & DELIVERY | SAMPLE INJECTION | COLUMN SELECTION | DETECTION | FRACTION COLLECTION

MATERIALS AND METHODS

In this application, an AZURA® Bio purification system consisting of AZURA P 6.1L HPG metal-free pump with 50 ml pump head; AZURA ASM 2.1L assistant module with UVD 2.1S detector and an injection valve; a bioinert multifunction selection valve; AZURA CM 2.1S conductivity monitor and Foxy R1 fraction collector was used. Prior to the run the cation exchange column (Sepapure SP FF6 1 mL) was equilibrated with buffer A (20 mM sodium phosphate buffer pH 6.1). The flowrate for the run was 1 mL/min. 100 µL protein mixture (α-Chymotrypsinogen A 0.33 mg/mL, Cytochrome C 0.33 mg/ml, Lysozyme 0.33 mg/mL) was injected. The column was washed with 5 mL buffer A to remove all unbound protein. The proteins were eluted with a linear gradient from 10 % buffer B (20 mM sodium phosphate buffer pH 6.1, 1 M NaCl) to 40 % B for 20 mL. The column was regenerated with a high salt wash of 5 mL buffer B 100 % followed by a re-equilibration of the column with 10 mL of buffer A at a flowrate of 2 mL/min. The proteins were detected at 280 nm and conductivity signal was recorded to monitor the salt gradient.

CONCLUSION

The principle of ion exchange chromatography was illustrated. Three model proteins eluted under increasing salt concentrations from the cation exchange column. The AZURA Bio purification system is well suited for all gradient methods like ion exchange, hydrophic interaction and reversed phase chromatography. Isocratic methods like size exclusion and affinity chromatograpy are as well supported. AZURA Bio purification system is the ideal system for your protein purification task.



ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

Eluent A	20 mM Sodium phosphate buffer pH 6.1		
Eluent B	20 mM Sodium phosphate buffer pH 6.1 + 1M NaCl		
Gradient	Volume [mL]	% A	% B
	0-5 step	100	0
	5-25 gradient	90	10
		60	40
	25-30 step	0	100
	30-40 step	100	0
Flow rate	1 mL/min from 25 mL: 2 mL/min	System pressure	0-1 bar
Column temperature	RT	Run time	40 min
Injection volume	100 µL	Injection mode	Injection valve
Detection UV	280 nm	Data rate	2 Hz

Tab. A2 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L, HPG 50 mL	APH68FB
Assistant	AZURA ASM 2.1 L Left: UVD 2.1S Middle: - Right: 6-Port 2-Pos 1/16", PEEK	AYCALXEC
Valve	Bioinert multifunction selection valve	AWB00FC
Flow cell	Semi-preparative, UV Flow cell, 3mm, 1/16", 2 µL volume, biocompatible	A4045
Conductivity monitor	AZURA CM 2.1S	ADG30
Flow cell	Preparative up to 100 mL/min	A4157
Column	Sepapure SP FF6 1 mL	010X15RSPZ
Fraction collector	Foxy R1	A59100
Software	Purity Chrom Basic	A2650

RELATED KNAUER APPLICATIONS

VBS0063 – Automated two – step purification of mouse antibody IgG1

VBS0064 – Comparison of IgG purification by two different protein A media

VBS0067 – Automated two-step purification of 6xHis-tagged GFP

VBS0069 – Purification of Sulfhydryl Oxidase

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

VBS0074 - Comparison of ion exchange columns

VBS0075 - Group separation with Sepapure Desalting on AZURA Bio purification system

