Science Together

KNAUER

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

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

SUMMARY

Ion exchange chromatography is one of the most widely used FPLC techniques for protein separation and purification. Depending on the charge of the sample and the resin cation or anion exchange chromatography is used. This application describes an easy separation of model proteins and explains how anion exchange chromatography works.

INTRODUCTION

Ion exchange chromatography separates molecules according to type and strength of their charge. The isoelectric point (pI) is the pH where a protein or molecule has no net electrical charge. Depending on the pH of the buffer a protein has different surface charges in solution. At a pH above their pI proteins have a negative charge and bind to positively charged resins such as anion exchangers **(Fig 1)**. This interaction is used for the separation and purification of various proteins. By using a suitable pH and low salt conditions proteins bind to the resin in the initial step. Proteins are mostly separated with a linear salt gradient whereby the salt ions compete with the proteins for bindings sites. Proteins with weak ionic interactions are the first to elute from the column. In the case of anion exchange chromatography, proteins that are less negativly charged start to elute first. With an increase of the salt concentration proteins with stronger ionic interaction elute later from the column. Ion exchange resins are categorized as strong or weak exchangers. Strong ion exchange resins are fully charged over a wide range of pH levels, while weak ion exchangers have depending on the pH varying ion exchange capacity. Weak ion exchangers have different selectivity's compared to strong ion exchangers. This application describes the separation of Conalbumin, α-Lactoglobin and soy bean Trypsin inhibitor on a weak and a strong anion exchanger and explains the principle of anion exchange chromatography.

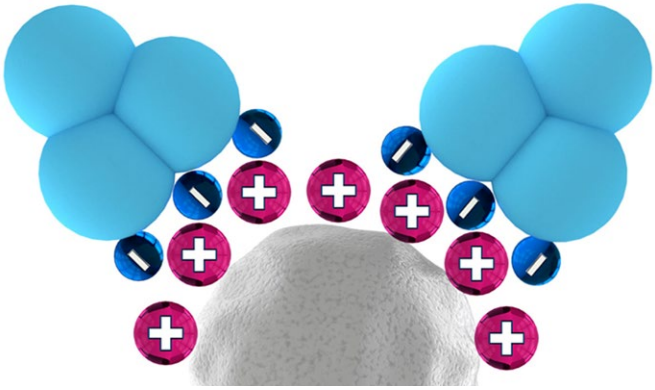


Fig. 1 Principle of anion exchange chromatography

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

RESULTS

Conalbumin (pI 6.8), α-Lactoglobin (pI 5.8), and soy bean Trypsin inhibitor Cytochrome C se A (pI 4.5) are proteins with relatively low pI values, which make them ideal candidates for anion exchange chromatography **(Fig 2 & 3)**. All three proteins bind under low salt conditions to the resin. Conalbumin A eluted first from the column due to its highest pI within the group of separated proteins. With an increasing gradient and therefore increasing salt concentration α-Lactalbumin eluted as second peak while soy bean Trypsin inhibitor eluted as third peak. The single protein standards were separated on each column to assign the peaks **(Fig 2. A & B)**.

The identical protein mix was run on a weak (light blue signal Sepapure DEAE) and strong (dark blue signal Sepapure Q) anion exchangers showing the different selectivity of these two resins.

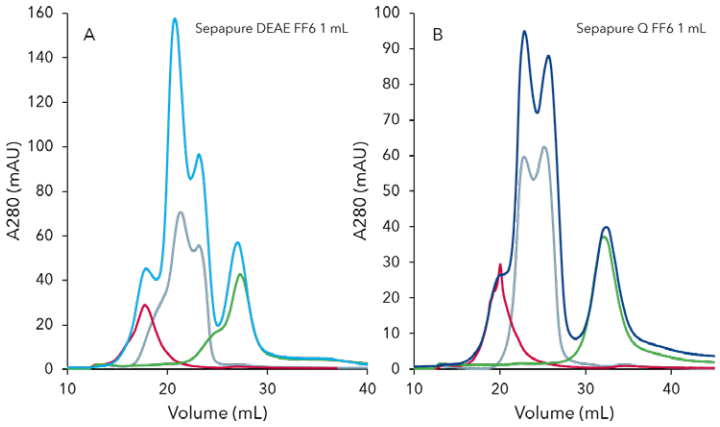


Fig. 2 Overlay of chromatograms on weak (A) and strong (B) anion exchange chromato-graphy columns. Conalbumin (red line), α-Lactalbumin (grey line) and soy bean Trypsin inhibitor (green line), sample mix light blue for weak (A) and dark blue for strong (B) anion exchange chromatography columns

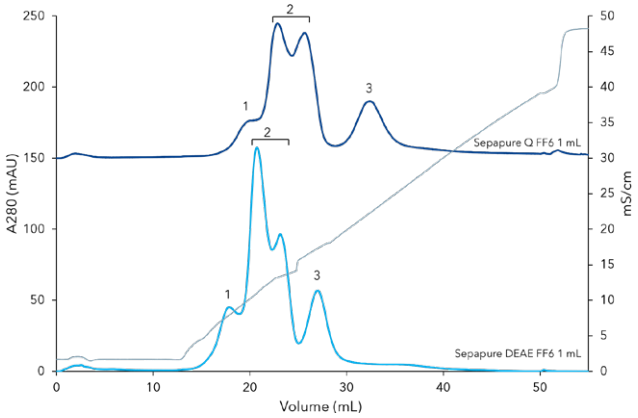


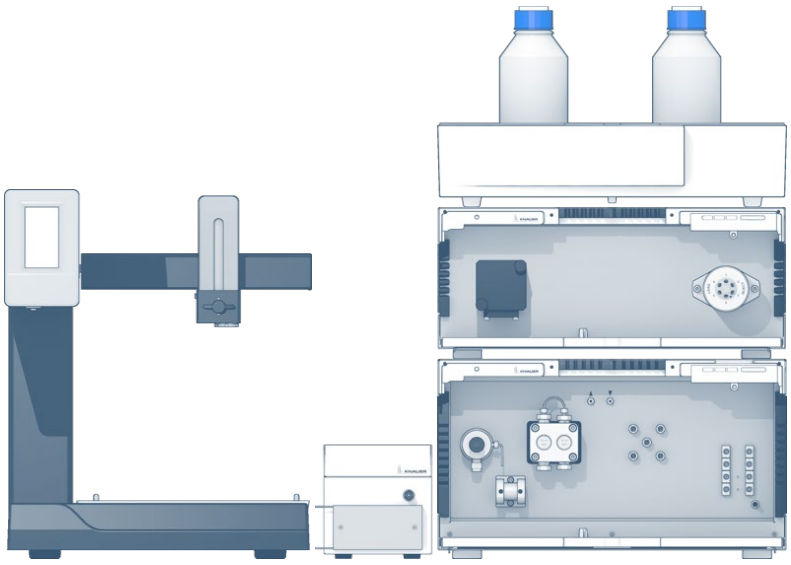
Fig. 3 Chromatograms of the separation of Conalbumin (1), α-Lactalbumin (2) and soy bean Trypsin inhibitor (3) with weak (light blue line) and strong (dark blue line) anion exchange chromatography columns, grey line: conductivity signal

MATERIALS AND METHODS

In this application, an AZURA Bio LC 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. Conalbumin (0.2 mg/mL), α-Lactalbumin (0.4 mg/mL) and soy bean Trypsin inhibitor (0.6 mg/mL) were diluted and mixed in buffer A (20 mM Tris/HCl pH 7.4) to the final concentration. Prior to the run the anion exchange columns (Sepapure Q FF6 1 mL and Sepapure DEAE FF6 1 mL) were equilibrated with buffer A. 2 ml of the sample was injected with a flowrate of 1 ml/min. The column was 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 40 % buffer B (20 mM Tris/HCl pH 7.4, 1 M NaCl). The proteins were detected at 280 nm and conductivity signal was recorded to monitor the salt gradient.

CONCLUSION

Three model proteins with different surface charges eluted under increasing salt concentrations from the anion exchange columns illustrating the principle of anion exchange chromatography. The application demonstrates the different selectivity of Sepapure DEAE, a weak, and Sepapure Q, a strong, anion exchange resin.



ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

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. A2 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 Positi-on 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	010X15HSPZ 010X15ISPZ
Fraction collector	Foxy R1	A2650
Software	Purity Chrom	A2680

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

VBS0074 – Comparison of ion exchange columns

