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Small-scale SEC with AZURA® compact FPLC

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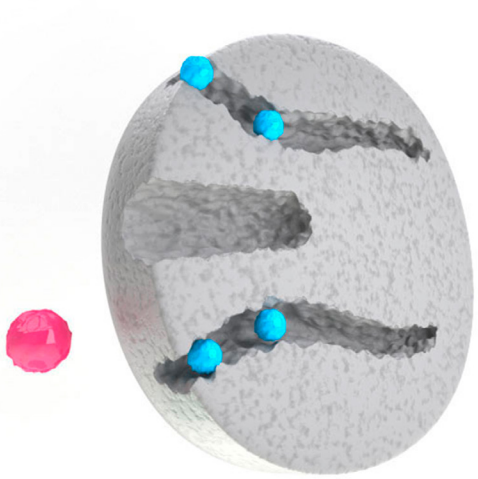

SUMMARY

Size exclusion chromatography (SEC) separates molecules depending on differences in their size. SEC is frequently used as a polishing step during protein purification and can also be used for the analysis of protein homogeneity. In recent years the use of small-scale SEC for the analysis of low sample volumes or for the purification of small sample amounts has become more important; especially due to the development of suitable columns. Here, we performed a proof-of-concept study to the evaluate the performance of the AZURA® compact SEC system for the use of small-scale SEC applications and describe the system configuration for this application.

INTRODUCTION

Size exclusion chromatography is a well-established liquid chromatography technique that uses a porous matrix to separate molecules based on differences in their size. The basic separation principle of SEC relies on the fact that smaller molecules are more likely to enter the pores of the column, meaning their time spent traveling through the column matrix is longer than that of larger molecules. Separations are facilitated by the selection of optimal particle and pore sizes, and the length of the column used. SEC is frequently used as a final or polishing step during protein purification where protein samples are screened for monodispersity. Inhomogeneous samples or partially aggregated samples may be an indication of improper folding and compromised activity. Additionally, the oligomerization state of the sample, which can be of biological importance, can be analysed using SEC [1, 2]. Small-scale SEC columns are now available that allow for the high-resolution analysis or small-scale purification of monoclonal antibodies (mAb) and other biomolecules. These columns can withstand higher pressure allowing them to be used at higher flowrates, which results in a shorter run time, whilst small particles and pores provide excellent resolution. Here we describe the use of a SEC compact system to be used with small-scale SEC columns.

Additional Information

Small-scale SEC with AZURA® compact FPLC

RESULTS

The compact AZURA Bio SEC system is designed especially for time-intensive SEC methods. For this application the capillaries of the system were adapted to fit the requirements of small-scale SEC. To prevent the possible dilution of the injected sample as it travels from the injection valve to the column and to avoid peak broadening at the column outlet, the PEEK capillaries were changed to 0.13 mm ID tubing **(see Fig. 1)**. Please note that if fractions are being collected, the tubing that connects to the fraction collector should also be changed.

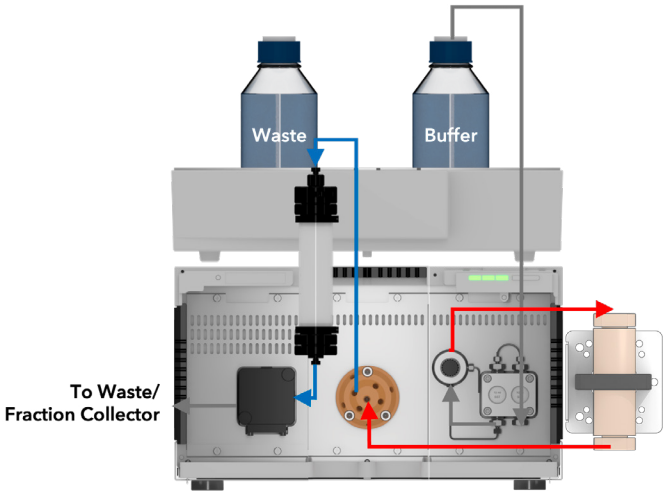

Fig. 1 System configuration with 1/16" PEEK capillary connections from pressure sensor/pump outlet to UV detector inlet, red tubing 0.25 mm ID, blue tubing 0.13 mm ID

The smaller the inner diameter of the tubing that is used, the higher the back pressure within the system. Care should be taken, as this back pressure could exceed the pressure limit of your column hardware or your system. As such, before proceeding further, we analysed the back pressure within the system without the column **(see Fig. 2)**. Two setups, one without a fraction collector **(Fig. 2 blue line)** and one with an additional 70 cm 0.13 mm ID capillary to the fraction collector **(Fig. 2 red line)** were tested. The used column (Superdex 200 Increase 3.2/300) was ran at typical flow rates of around 0.07 ml/min with a maximum flow rate of 0.15 ml/min at room temperature. The maximum pressure limit of the column hardware and the system is 50 bar. The typical pressure drop over the packed bed at a maximum flow rate of 0.15 ml/min at room temperature is 20 bar. Both setups can be used with the Superdex 200 Increase 3.2/300.

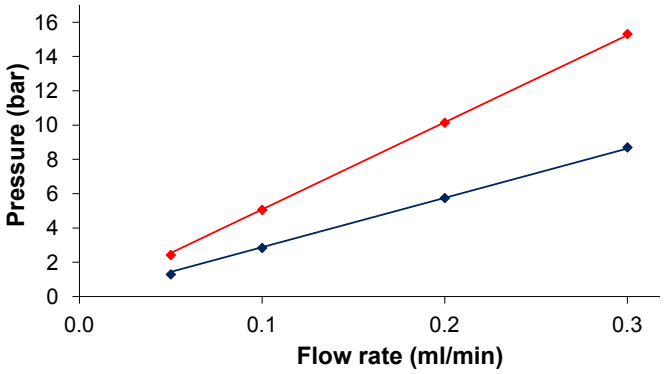

Fig. 2 Systemic pressure versus flow rate of the AZURA compact system (blue) pressure with capillary connections as described in Figure 1; red with additional 0.13 mm ID tubing to fraction collector

Following this, a gel filtration standard was tested to assess the ability of the system for small-scale SEC. The chromatogram shows excellent separation of the standards **(Fig. 3)**. The flow rate was confirmed with a flow meter. The measured average systemic pressure with a column was 11.7 bar without a fraction collector and 13.2 bar with a fraction collector. The pressure fluctuation was below 1 bar (data not shown).

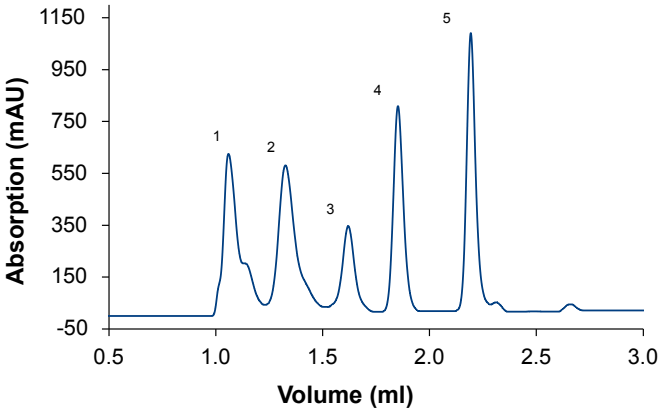

Fig. 3 Chromatogram of gel filtration standard; (1) Thyroglobulin; (2) gamma-Globulin (3) Ovalbumin; (4) Myoglobulin; (5) Vitamin B12

CONCLUSION

The AZURA compact SEC system can be easily modified with smaller capillaries to optimize the systemic dead volume to a minimum for small-scale applications. With these changes the flow rate for the tested application was precise, the back pressure was below the systemic pressure limit and the pressure fluctuation was low. The chromatogram of a SEC standard showed a satisfactory separation. We can recommend the use of AZURA compact SEC system for small-scale SEC applications.

MATERIALS AND METHODS

System configuration

Instrument	Description	Article No.
AZURA SEC FPLC System	Isocratic FPLC System for Size Exclusion Chromatography (SEC) for up to 10 ml/min	SYS872101122
Tubing	1/16" Peek capillary, 0.13 mm ID	A2522
	1/16" Peek capillary, 0.25 mm ID	A2524
Column	Superdex 200 Increase 3.2/300	n.a.

The flow rate was measured with a Mini CORI-Flow (M12) flowmeter (A5394).

Analytes of the gel filtration kit

Analyte	Concentration	MW	Source
Thyroglobulin	5 mg/ml	670000 Da	Bovine thyroid
gamma-globulin	5 mg/ml	158000 Da	bovine
Ovalbumin	5 mg/ml	44000 Da	chicken
Myoglobin	2.5 mg/ml	17000 Da	equine
Vitamin B12	0.5 mg/ml	1350	

Method parameters

Parameter	Description
Buffer	PBS (0.01 M phosphate buffer; NaCl 0.138 M; KCl – 0.0027 M; pH 7.4, at 25 °C)
Flow rate	0.07 ml/min
Gradient mode	isocratic
Temperature	ambient
Detection	UV 280nm
Injection volume	20 µl*
Sample loop volume	10 µl
Data rate	10 Hz
Run time	20 min

*The multi-injection valve has an internal port volume of 10 µl, resulting in a total injection volume of 20 µl (10 µl ports + 10µl sample loop)

REFERENCES

[1] Wingfield PT. Overview of the purification of recombinant proteins. Curr Protoc Protein Sci. 2015;80:6.1.1-6.1.35. Published 2015 Apr 1. doi:10.1002/0471140864.ps0601s80

[2] Structural Genomics Consortium., Architecture et Fonction des Macromolécules Biologiques., Berkeley Structural Genomics Center. et al. Protein production and purification. Nat Methods 5, 135–146 (2008). https://doi.org/10.1038/nmeth.f.202