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Fast protein analysis of mucins using an AZURA® SEC System

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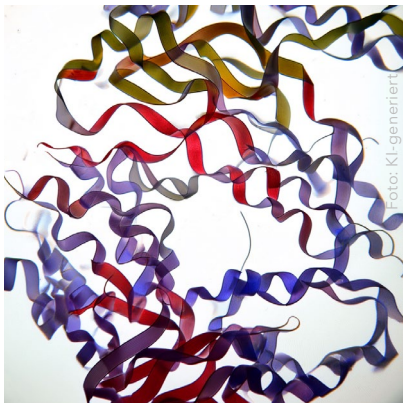

SUMMARY

The KNAUER AZURA® SEC System is known as a robust **G**el **P**ermeation **C**hromatography (GPC) or **S**ize **E**xclusion **C**hromatography (SEC) system for advanced applications as well as high-throughput analysis. In combination with an AppliChrom® VivoSep column we demonstrate the performance of the KNAUER AZURA® SEC System for the analysis of commercially available bovine submaxillary mucin and porcine gastric mucin. The VivoSep SEC column material uses a multi-stage, hydrophilic modified polymer-based design that enables high-resolution analyses of proteins over a wide molecular weight range.

INTRODUCTION

Mucins are glycoproteins, which are characterised by a combination of proteins and carbohydrates[1].

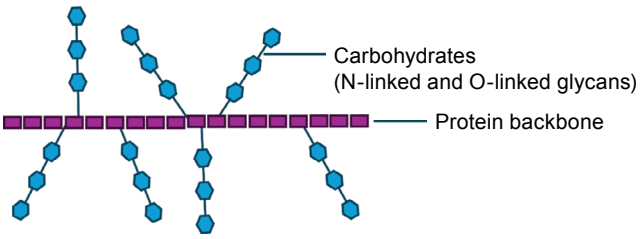

Fig. 1 Model of the glycoprotein structure of mucins.

They play an important role in biological processes, particularly in the protection of mucous membranes against mechanical, chemical and biological influences[2]. Due to their high content of sugar molecules, mucins have a strong hydrophilic tendency, which helps them to bind water and form a gel-like consistency[3,4,5]. They are part of a mixture of water, salts, lipids, cell material and other proteins that form the mucus that covers the surfaces of the mucous membranes of organisms such as the mouth and nose[1,2]. Thereby, the mucins are crucial for the physical properties of the mucus, especially its viscosity[1].

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An important analytical tool for the characterisation of mucins is SEC[1]. This method allows the determination of the molecular weight and molecular weight distribution of mucins, contributing to a better understanding of their biological functions[1]. In addition, SEC allows efficient isolation of mucins from biofluids, resulting in purified mucin fractions that can be used for further analysis or medical applications, such as biomarkers in cancer diagnosis and prognosis[6,7]. Overall, SEC is a gentle chromatographic technique performed under mild conditions such as room temperature and neutral pH[8]. This ensures that the native conformation and function of the mucins are maintained throughout the separation process, which is crucial for preserving their biological activity and interactions[8].

SAMPLE PREPARATION

Commercially available bovine submaxillary mucin and porcine gastric mucin samples were used for the analysis.

RESULTS

The most commonly used materials in SEC include cross-linked dextran or agarose (Sephadex/Sepharose), polyacrylamide beads (Bio-Gel), and dextran derivatives (Sephacryl)[9]. AppliChrom® has developed the VivoSep SEC series which enables high resolution separations of proteins. This special multistage hydrophilic modified polymer-based SEC column offers significantly expanded capabilities for analysing larger proteins by covering a molecular weight range from 100 Da to 5 000 000 Da. Here, we demonstrate that this column material, in conjunction with the KNAUER AZURA® SEC System, is suited for straightforward measurements of and porcine gastric mucin **(Fig. 2)** and bovine submaxillary mucin **(Fig. 3)**. The shown data for this application note were provided by AppliChrom®.

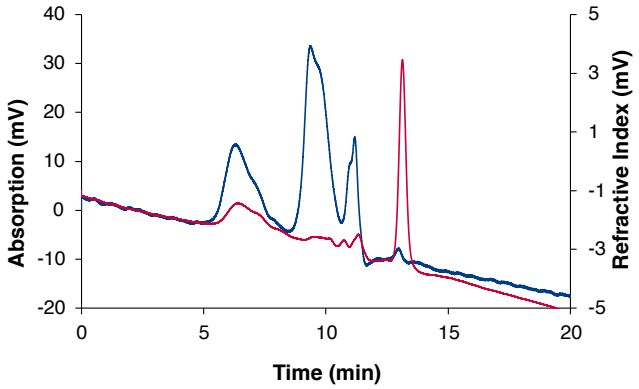

Fig. 2 Chromatogram of the porcine gastric mucin measured with the VivoSep SEC 350 (separation range 2 500 - 1 000 000 Da), DAD at 220 nm (blue) and RID (red).

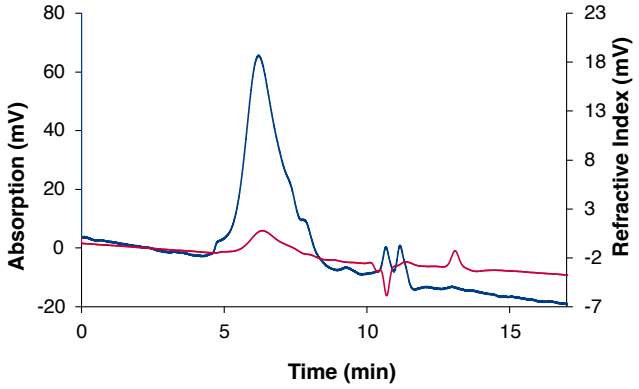

Fig. 3 Chromatogram of the bovine submaxillary mucin measured with the VivoSep SEC 350 (separation range 2 500 - 1 000 000 Da), DAD at 220 nm (blue) and RID (red).

It has been shown that mucins can be analysed in 15 minutes **(Fig. 2** and **Fig. 3)**. In contrast, analysis using common columns with cross-linked dextran or agarose based resins require up to 300 minutes[10]. In addition, AppliChrom® VivoSep SEC columns have an excellent pressure stability of 50 to 200 bar, depending on the pore size. In comparison, cross-linked dextran SEC columns are only stable at pressures of 5 bar or less. This high pressure stability also allows the use of smaller particles (7 - 10 µm), which results in enhanced separation performance.

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CONCLUSION

The KNAUER AZURA® SEC System in combination with the AppliChrom® VivoSep SEC column offers significant time savings for the analysis of mucins. This solution provides good separation performance and is an innovative addition to the field of protein size exclusion chromatography.

MATERIAL AND METHODS

Tab. 1 Method parameter.

Parameter	Value
Flow rate	1 ml/min
Isocratic	H_2O / PBS-Buffer
Column temperature	25 °C
Injection volume	8 µl
Injection mode	Partial loop
Detection 1	DAD 220 nm
Detection 2	RID
Data rate	10 Hz
Time constant	0.1 s
Time	20 min

Tab. 2 System configuration.

Instrument	Description	Article No.
Pump	AZURA® P 6.1L LPG Pump with 10 ml pump head, stainless steel	APH30EA
Autosampler	AZURA® AS 6.1L, analytical HPLC autosampler, 862 bar	AAA50AA
Detector 1	AZURA® DAD 2.1L, 190 - 700 nm	ADC01
Detector 2	AZURA® RID 2.1L, analytical refractive index detector	ADD31
Thermostat	AZURA® CT 2.1	ATC00
Eluent tray	AZURA® E 2.1L	AZC00
Column	AppliChrom® VivoSep SEC 350, 10 µm, 300 x 8 mm, 2 500 - 1 000 000 Da	30GN-46BABN
Capillaries	Start-Up Kit with flexible, precut capillaries for analytical HPLC systems with 1/16" connections	AZF120
Software	ClarityChrom® 9.1.0 - Workstation, autosampler control included	A1670
Software	ClarityChrom® 9.1.0 - SEC/GPC extension	A1678

Fig.4 SEC system setup.

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