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Application Note

Automated purification of human antibodies (IgG) on AZURA Bio LC

Category	Bio purification
Matrix	Blood, Serum
Method	FPLC
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Analytes	Serum, antibodies
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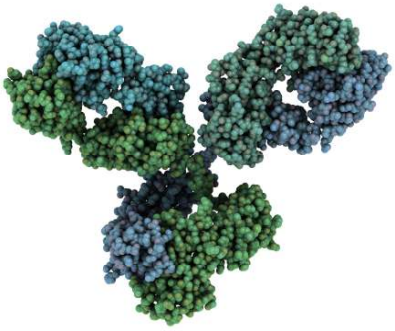


Summary

This application note describes an automated purification of human antibodies (IgG) from blood serum. The antibodies were first purified with a protein G affinity chromatography column and subsequently applied on a gel filtration column to exchange the buffer conditions. This application note highlights the possibility of automated purification with the AZURA BIO LC system without manual interaction during the course of the purification. Peak parking of the elution peak from the affinity chromatography method and re-injection of the sample onto the gel filtration column are described in detail.

Introduction

Antibodies (IgGs) are part of the immune system. They can identify and bind particular antigens thereby neutralizing them. Due to their specific target recognition/binding function they are tremendously important in the biotechnology and pharmaceutical industry. They can be used for the diagnosis and treatment of diseases. Besides, antibodies are also the key components in numerous research applications such as Western Blots and Immunoassays. Quality and purity of the IgG is important for these applications. Antibodies are usually purified by several steps with manual interaction.

The aim of this application note was to establish an easy automated purification method on the AZURA Bio LC purification system combining an affinity chromatography step with a gel filtration step to exchange the buffer of the purified antibodies. This application note is an example of a time-saving automation of protein purification and can be easily adapted to several protein purification protocols.

Sample preparation

500 µl of human blood serum were used for the purification of IgG.

Method setup

The system configuration and the different valve settings are shown in figure 1. The first injection valve (V1) is used for the injection of the sample. The other two injection valves are necessary for the inversion of the flow direction (V2 + V3). They also switch the flow to the waste/fraction collector and in/out of the Superloop™*. The Superloop allows the collection and (re)injection of large sample volumes into a pressurized system. The two multi-port valves (V4 + V5) are used for column switching. In the initial configuration (Fig. 1A) the sample is injected onto the column. The non-binding protein is directed to the waste. After washing the column, IgG is eluted with eluent B (Fig. 1.B). The valves are switched to the peak parking position (V2 + V3 in Load position). The eluting protein is collected in the Superloop. Subsequently the collected protein is automatically re-injected onto the second column by changing the valve position for V2 and V3 from Load to Inject and for V4 and V5 from position 1 (column 1) to position 2 (column 2) (Fig. 1C). The flow is inverted and the Superloop is emptied. The eluted protein peak is fractionated by a fraction collector. No manual interaction is necessary during the purification.

Method parameters

Parameter	Description
Column 1:	Affinity chromatography: Hi Trap Protein G, 5 ml
Column 2:	Gel filtration: BioFox 40/1200 SEC, 300 x 8 mm ID, 15 ml
Eluent A	20 mM sodium phosphate buffer, pH 7.5
Eluent B	Glycin-HCl, pH 2.7
Eluent C	20 mM sodium phosphate buffer, pH 7.5
Injection volume	500 µl human serum
Column temperature	ambient
Detection	UV at 280 nm, conductivity, pH
Run time	96 min

Method	Time	Gradient	Flow rate	Description
Affinity chromatography	0 – 26 min	100% A	0.5 ml/min	Injection and Washing
	26 – 46 min	100% B	2 ml/min	Elution and Peak- Parking
Gel filtration	46 – 86 min	100% C	1 ml/min	Reinjection
Affinity chromatography	86 – 96 min	100% A	2 ml/min	Re-equilibration

Fig. 1

System configuration and flow chart of the FPLC system

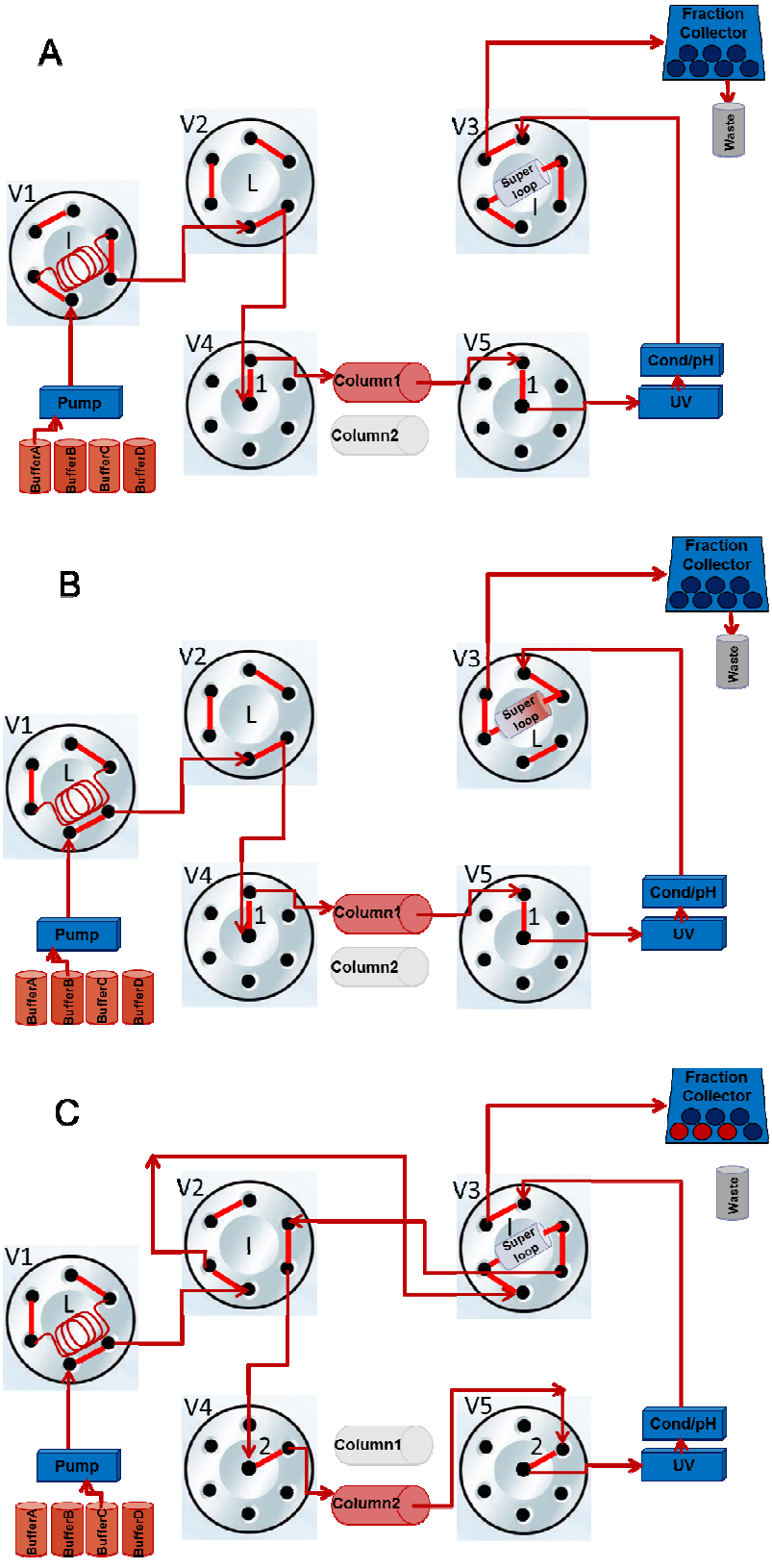
A. Initial configuration

B. Protein parking

C. Re-injection

L = Load position

I = Inject position



Results

Human antibodies were purified from human serum by Protein G affinity chromatography. The eluted protein was automatically collected and then re-injected onto a gel filtration column and fractionated. Figure 2 depicts the chromatogram of the automated purification of the human IgG from blood. The first box shows the injection of the serum and washing of the column. Peak 1 reflects all unbound contaminating protein. In the second box the bound IgG is eluted by a strong acidic pH (peak 2) and parked in the Superloop. The collected IgG is re-injected onto the gel filtration column to exchange buffer conditions (Fig.2. Box 3). IgG elutes as one peak (peak3). Peak 4 is the glycine buffer peak. The peaks from the gel filtration column were fractioned and analyzed by SDS-PAGE. Finally the protein concentration was determined.
The purity of the human IgG was estimated by SDS-PAGE. Human IgG has a molecular weight of 144 kDa[1]. It has two light (each 22 kDa) and two heavy (each 50 kDa) chains. The SDS-PAGE showed two distinct bands according to the molecular weight of the heavy and the light chain of IgG (Fig. 3). With this automated method 3.48 mg of pure IgG could be obtained out of 500 µl of human serum.

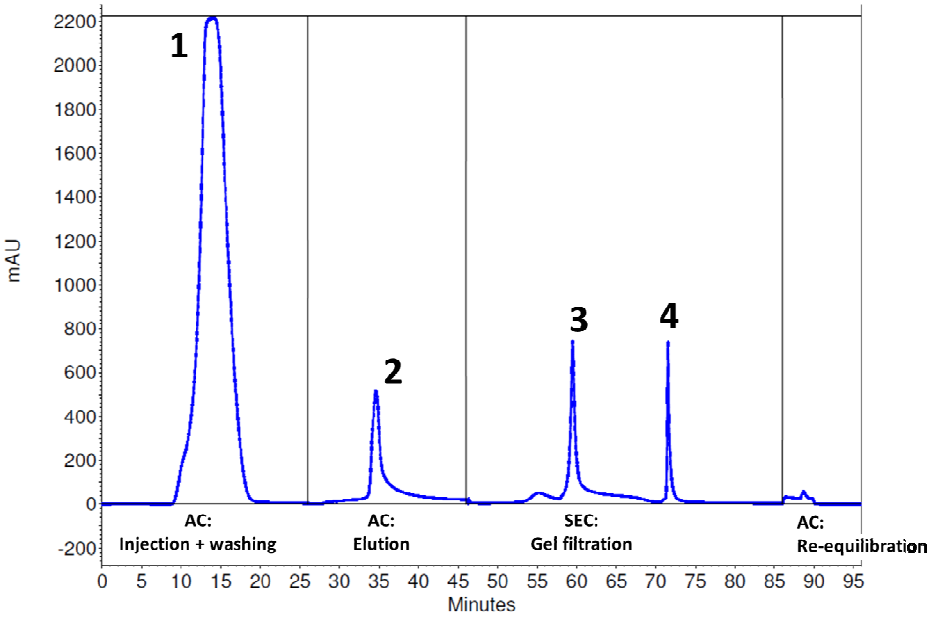


Fig. 2

Chromatogram of the IgG purification and the corresponding method steps

1. Injection peak of human serum
2. Elution peak of IgG from AC column, parked in Superloop
3. Elution peak of IgG from gel filtration column
4. Glycine buffer peak

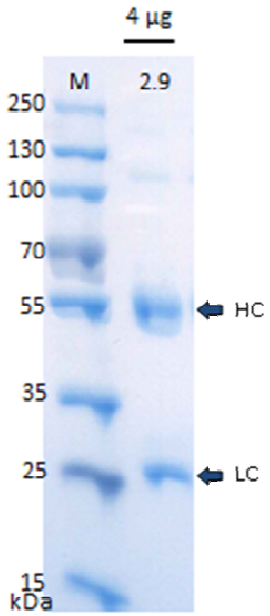
Fig. 3

SDS-Page of the eluted IgG (peak 3)

M: protein marker in kDa

HC: heavy chain, 50 kDa

LC: light chain, 22 kDa



Conclusion

Human IgG could be successfully purified by an automated combination of an affinity chromatography and gel filtration method on the new AZURA Bio LC system. No manual interaction was necessary. The method setup could easily be adapted to other purification protocols for the separation of biomolecules.

References

1. Janeway CA Jr, Travers P, Walport M, et al.; Immunbiology: The Immune System in Health and Disease. 5th Edition, New York; Garland Science, 2001

Authors

Dr. Ulrike Maschke, Biochromatography, Columns and Applications Department, KNAUER

Dr. Yannick Krauke, Biochromatography, Columns and Applications Department, KNAUER

Properties of recommended columns

Stationary phase	HiTrap Protein G
Matrix	Agarose
Binding capacity	25 mg human IgG/ column
Ligand	Recombinant protein G lacking albumin-binding region
Maximum pressure	5 bar
Particle size	34 µm
Column dimension	25 x 7 mm ID
Volume	1 ml

Stationary phase	BioFox 40/1200 SEC
Matrix	Agarose
Exclusion limit	1200 kDa
Particle size	40 µm (32-60 µm)
Maximum Pressure	40 bar (column 20 bar)
Agarose content	7.4 – 4.8 %
Column dimension	300 x 8 mm ID
Volume	15 ml
Order Number	30GX46KBFZ

Recommended instrumentation

The automated purification was performed on a biocompatible Knauer AZURA Bio LC system with a low pressure gradient pump, a UV detector, a conductivity monitor, a fraction collector.

Title	Description	Art. No
AZURA P6.1.L	Quaternary Pump + degasser, metal-free, 10 ml pump head	APH64EB
AZURA UV/VIS Detector UVD 2.1L	190-750 nm variable single wavelength UV/VIS detector	ADAO1XA
Analytical Flow Cell UV	3 mm path length, 1/16", 2 µl volume, 1 mm ID, PEEK	A4045
AZURA CM 2.1S	high precision online monitor for measurement of conductivity and optionally pH-value	ADG30
Flow Cell CM 2.1S, preparative	100 ml, 1/16", 0,75 mm ID, 100 bar for AZURA CM 2.1S	A4157
pH measuring kit	for conductivity monitor	A70091
AZURA Assistant ASM 2.1L	Sample injection assistant ASM 2.1L with Pump with pressure sensor, 50 ml pump head, titanium, 1 x injection valve, 1/16", PEEK 1 x 6 Mpos valve, 1/8", PEEK	AYBFECEL
AZURA Assistant ASM 2.1L	Column switch assistant ASM 2.1L with 6 port multi position valve V 2.1S, 1/16", PEEK, 6 port multi position valve V 2.1S, 1/16", PEEK, 6 port multi position valve V 2.1S, 1/8", PEEK,	AYEIEIEL
AZURA Assistant ASM 2.1L	Peak parking assistant ASM 2.1L with 6 port injection valve V 2.1S, 1/16", PEEK, 6 port injection valve V 2.1S, 1/16", PEEK, 6 port multi position valve V 2.1S, 1/8", PEEK,	AYECECEL
Fraction collector FOXY R1	max. 25 ml/min, 1/16" connectors, rack with 13 mm or eligible rack	A59100
OpenLAB CDS EZChrom Edition	Basic Workstation license, includes System Suitability, Fraction Collector Control and Software Maintenance Agreement	A2600-1

*) Superloop is a trademark of GE Healthcare Companies

Contact information

KNAUER
Wissenschaftliche Geräte GmbH
Hegauer Weg 38
14163 Berlin, Germany

Tel: +49 30 809727-0
Fax: +49 30 8015010
E-Mail: info@knauer.net
Internet: www.knauer.net