Evaluation of preparative icIEF with MS offline coupling for the analysis of charge variants of monoclonal antibodies

Antonia Wittmann[1], Yannick Wilke[1], Nicolas Grammel[2], Hermann Wätzig[1] I [1]Technische Universität Braunschweig, Institute of Medicinal and Pharmaceutical Chemistry I [2]Alvotech Hannover GmbH

antonia.wittmann@tu-braunschweig.de | Telephone +49 (0) 531 391-2777

Technische Universität Braunschweig

Wätzig et al.
Institute of Medicinal and Pharmaceutical Chemistry

Introduction

- Coupling icIEF (Imaged Capillary Isoelectric Focusing), an established technique for the analysis of charge variants of monoclonal antibodies, with MS (Mass Spectrometry) enables in-depth structural characterization. icIEF-MS therefore makes an important contribution to the evaluation of the product characteristics of biologics. [1]
- The general suitability of a procedure in which analytical icIEF methods are converted into a preparative method by increasing the sample concentration and adding 20 mM L-arginine as a cathodic spacer was tested with Matuzumab and Infliximab (IgG1 each) [2], [3]. After chemical mobilization and fraction collection, the identity of the Matuzumab fractions was determined by fluorescence measurement and reinjection of the protein-containing fractions, using the previously developed analytical icIEF method. [4]
- Mass spectrometry was subsequently performed. Chemical mobilization should prevent the transfer of icIEF reagents such as urea or methylcellulose into the fractions to a significant extent and is a different approach to other icIEF-MS concepts, e.g. based on the development of MS-compatible icIEF methods. [1]

Methods

Instrumental parameters	Analytical icIEF	Preparative icIEF
Instrument	MauriceFlex™, ProteinSimple, Bio-Techne (San Jose, CA, USA)	
Injection	55.0 s, by vacuum	20.0 sec, by vacuum
Automatic calibration	with fluorescence calibration standard before sample measurements	
Temperature controll	cooled at around 10 °C (default settings)	
Cartridge and capillary dimensions:	Maurice icIEF cartridge (capillary length: 50 mm, 100 µm id × 200 µm od, fused silica coated with fluorocarbon)	Maurice Flex icIEF fractionation cartridge (capillary length: 50 mm, 320 µm id, fused silica coated with fluorocarbon)
Detection: Fluorescence	10 sec exposure time 280 nm Exz. 320-450 nm Em.	0.2 sec exposure time 280 nm Exz. 320-450 nm Em
Voltage programm ([a]Infliximab & [b]Matuzumab)	[a]1.0 min 1500 V, 12.0 min 3000 V [b]1.0 min 1500 V, 11.0 min 3000 V	[a]10.0 min 500 V, 10.0 min 1000 V, 35.0 min 1500 V [b]10.0 min 500 V, 10.0 min 1000 V, 30.0 min 2000 V
Anolyte	80 mmol/l phosphoric acid in 0.1% MC	
Catholyte	100 mmol/l NaOH in 0.1% MC	
Data Evaluation	Compass for iCE 4.0.0 and 4.0.1	

Sample conposition analytical icIEF	
0.2 mg/ml Matuzumab	0.2 mg/ml Infliximab
Urea 2 mol/l	Urea 3 mol/l
0.35 % MC	0.35 % MC
Pharmalyte 5-8 2 %	Pharmalyte 5-8 3 %
Pharmalyte 8-10,5 2 %	Pharmalyte 3-10 1 %
pI-marker 9.5 & 6.14 2 % each	pI marker 8.40 & 6.14 1 % each
Ultrapure water 0.055 µS/cm	

Final concentrations are given, % in (v/v) MC (Methylcellulose), pI (isoelectric point)

Preparative icIEF

Modification of sample composition:
- Addition of L-arginin (20 mmol/l)
- Increased antibody concentration:

0.8 mg/ml Infliximab & 1.2 mg/ml Matuzumab

Fractionation of Matuzumab

- Chemical Mobilisation: 1000 V, 25.0 min, 5 mmol/l ammonium acetate, fraction colllection: 1000 V, 45.0 sec per fraction, in 5 mmol/l ammonium acetate, no refocusing

Verification experiments

- Identical icIEF analytical separation conditions (voltage programm and sample reagents)
- Concentration of the reference (non-fractionated Matuzumab): 0.05 mg/ml
- 1:5 dilution of the re-injected samples from fractionation
- Detection: native fluorescence, 100 sec exposure time

Results

Comparison between analytical and preparative icIEF separation

Figure 1
Electropherogramms of Matuzumab: analytical iciEF (left), preparative icIEF (right)

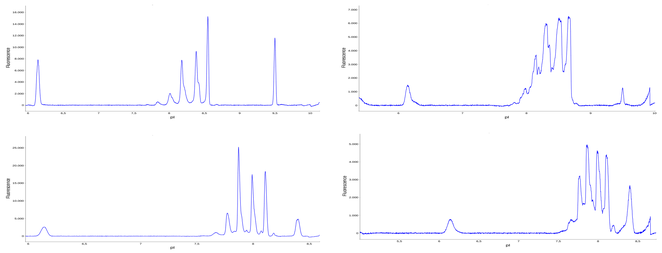
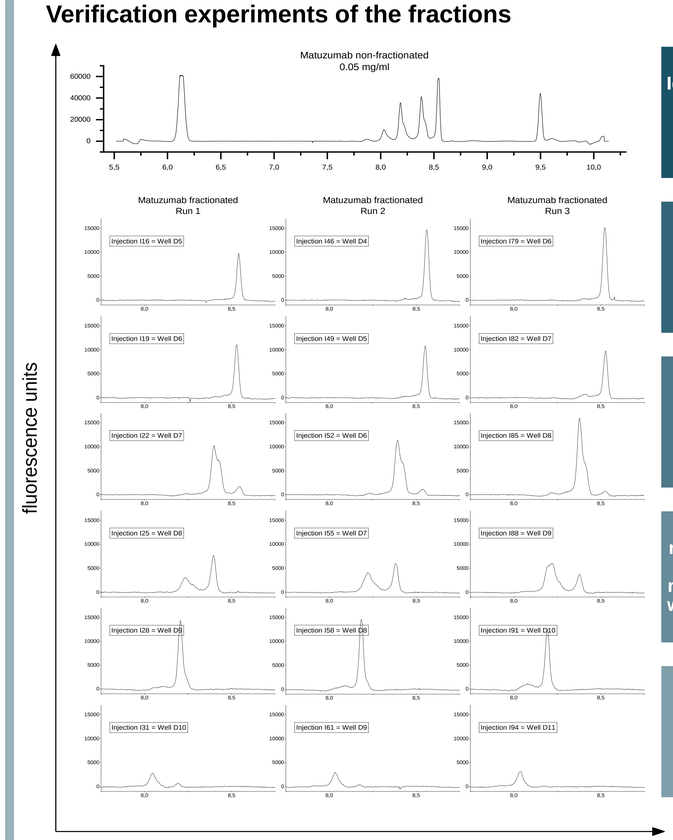


Figure 2
Electropherogramms of Infliximab: analytical iciEF (left), preparative icIEF (right)

Verification experiments of the fractions



Identical number of peaks and visually comparable peak profile of analytical and preparative icIEF

Preparative separation of Matuzumab: 50 minutes

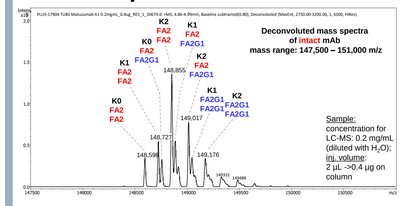
The entire process (separation & fractionation) requires approx. 2.5 hours.

Verification of a threefold repetition of a fractionation of matuzumab showed a recovery of the main isoforms with comparable peak profiles and positions per fraction.

Verification of fractions by analytical icIEF: 1-2 days depending on the number of fractions & repetitions of icIEF measurement

Figure 3
The same Matuzumab sample was separated (preparative mode) three times and collected each time in a new plate with fresh mobilizer solution. Several fractions of the three fractionations were each verified in triplicate using analytical icIEF (only one of three runs is shown).

Mass spectra of Matuzumab



Glycan structure	Oxford naming	Traditional naming
	FM0	Man3F
	M3	Man3
	FA1	G0F-N
	A1	G0-N
	FA1G1	G1F-N
	A1G1	G1-N

Glycan structure	Oxford naming	Traditional naming
	FA2	G0F
	A2	G0
	FA2G1	G1F
	A2G1	G1
	FA2G2	G2F
	A2G2	G2

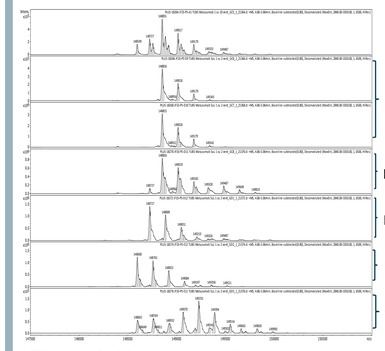


Figure 5: Legend for MS spectra labels: Labeling of N-glycan structures

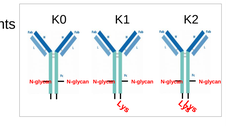


Figure 6: Legend for MS spectra labels: Lysine variants

Figure 4: Mass spectra Matuzumab non-fractionated (top) & Matuzumab fractions after preparative icIEF (below)

Conclusion and Outlook

- Transfer of analytical icIEF methods with common icIEF reagents to the preparative mode, demonstrated for Matuzumab and Infliximab, is easy to implement.
- The general suitability of preparative icIEF with MS offline coupling was demonstrated using Matuzumab.
- The preliminary results with Infliximab showed that the required sample concentration needs to be investigated in more detail.
- Selectivity, analysis time, reproducibility and sensitivity will be optimized in the future.

References

[1] Schlecht J, Moritz B, Kiessig S, Neusüß C. Characterization of therapeutic mAb charge heterogeneity by iCIEF coupled to mass spectrometry (iCIEF-MS). *Electrophoresis*. 2023;44(5-6):540-548.

[2] Deveuve Q, Lajoie L, Barrault B, Thibault G. The Proteolytic Cleavage of Therapeutic Monoclonal Antibody Hinge Region: More Than a Matter of Subclass. *Front Immunol.* 2020;11:168.

[3] Hartmann JT, Kollmannsberger C, Cascorbi I, et al. A phase I pharmacokinetic study of matuzumab in combination with paclitaxel in patients with EGFR-expressing advanced non-small cell lung cancer. *Invest New Drugs*. 2013;31(3):661-668.

[4] ProteinSimple, User Guide for Maurice, Maurice C., Maurice S. and MauriceFlex, Revision D, PN 046-295, 2023

Acknowledgement

Thanks to ProteinSimple (Bio-Techne) for providing their instrument and support (Finja Krebs, Udo Burger, Susanne Doerks) .

Thanks to Alvotech for mass spectrometry measurements and data analysis.