A robust method for chemical release and analysis of protein-bound N-glycans via horizontal fluorescence assisted carbohydrate electrophoresis in a HPE™ Blue Horizon™ cooled flatbed chamber

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Protein bound N-glycans are a major constituent of manufactured therapeutic glycoproteins. Due to variations in host cell performance during upstream processing at production scale the resulting glycoprotein therapeutics are characterized by N-glycan moieties composed of varying numbers of glycosidically linked hexose and deoxyhexose units. This heterogenic profile of N-glycan compositions within a manufactured batch of bulk drug substance affects the overall efficacy of the biologic drug and is therefore considered to be a critical quality attribute that needs to be monitored throughout production and prior to product release. Process analytical technology for assessing N-glycan composition in an industrial context not only requires affordable costs per measure point but also rugged equipment and rapid, robust, easy to implement methods that ensure reliable long-term performance of the glycan analysis system in a process critical setting.

Here we describe an analytical solution that facilitates the time- and cost-efficient processing of multiple glycan samples in a single run. This newly developed chemical glycan release procedure is based on a system of stable reactants that are able to generate and recycle the highly reactive electrophilic glycan release agent in-situ. C18- reversed phase and HILIC-HPLC-FLD analysis revealed the N-glycan profiles of the chemically released N-glycans to be consistent with those obtained by standard PNGase F treatment. Time and cost reduction in sample preparation and analysis is achieved by elimination of an otherwise essential sample cleanup step combined with the direct analysis of multiple glycan preparations (n=24) in a single separation run. The direct analysis of fluorescently labelled glycans on a horizontal gel electrophoresis system with lot controlled precast gels and validated methodology allows for consistent and accurate characterization and quantification of large sets of incurred samples from biomanufacturing runs and circumvents the need for laborious sample clean-up.

High Resolution Electrophoretic Separation of labelled Glycans in an evenly chilled, ultrathin polyacrylamide matrix

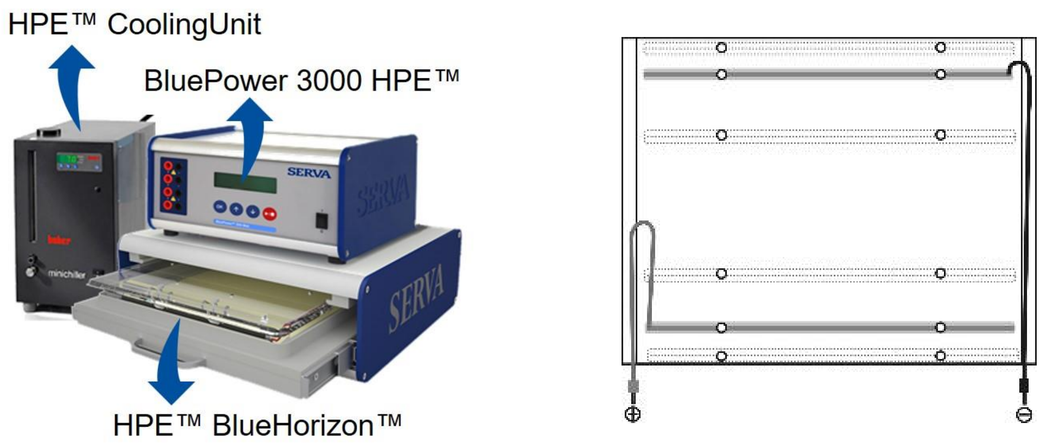


Fig. 1: Complete HPE™ Blue Horizon™ System including power supply, chiller, cooled flatbed chamber and horizontal gel assembly (right)

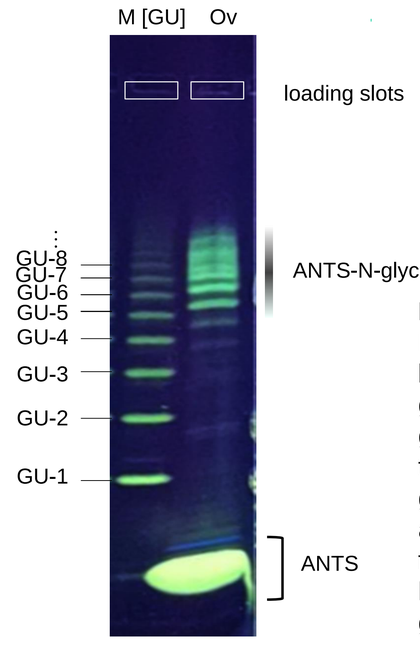


Fig. 2: Blue Horizon™ FACE (BHZ-FACE)
Lane M: Standard Dextran partial hydrolysate ANTS labelled; Lane Ov: ANTS-N-glycans of the test glycoprotein ovalbumin separated by BHZ-FACE. GU= glucose unit of the dextran standard. The GU-1 band of the standard corresponds to fluorescently labeled glucose. Note the selective removal of a dominant amount of excess ANTS fluorescent label molecules from the N-glycan fraction. BHZ-FACE has a uniquely high capability to remove excess label from labelled glycan samples.

Chemical glycan release by in-situ generated and recycled hypobromite

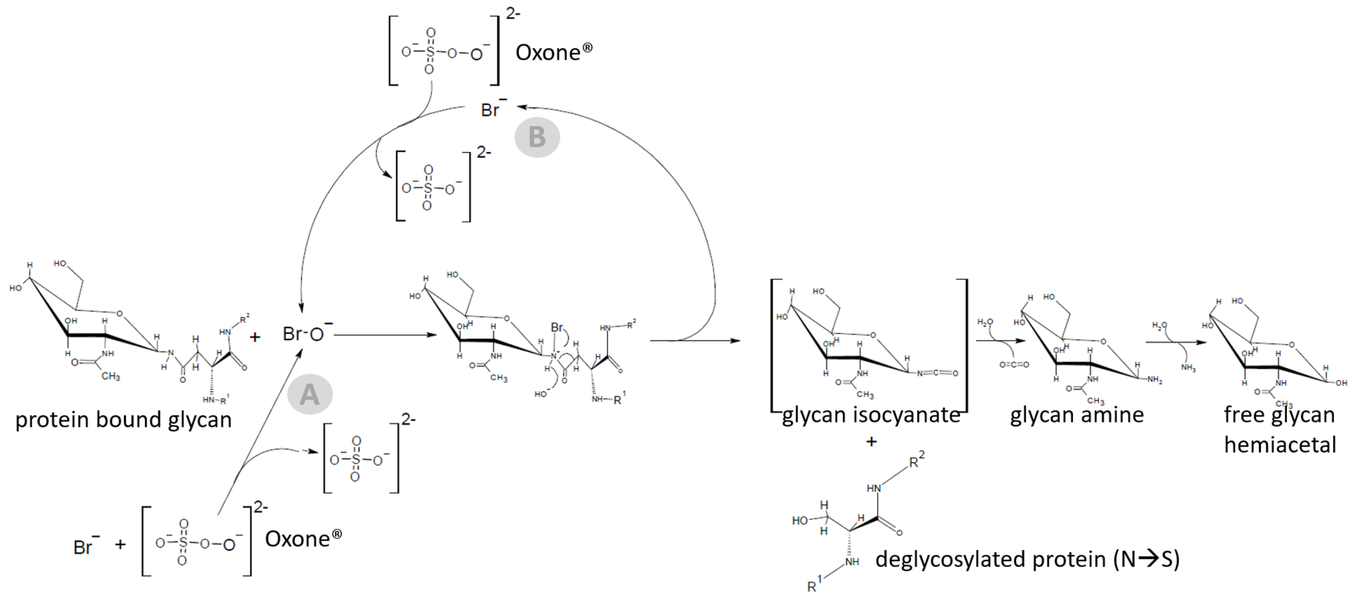


Fig. 3: Proposed mechanism for cleavage of the Asparagin-N-glycan carboxamide group by hypobromite generated in-situ from Oxone® and bromide

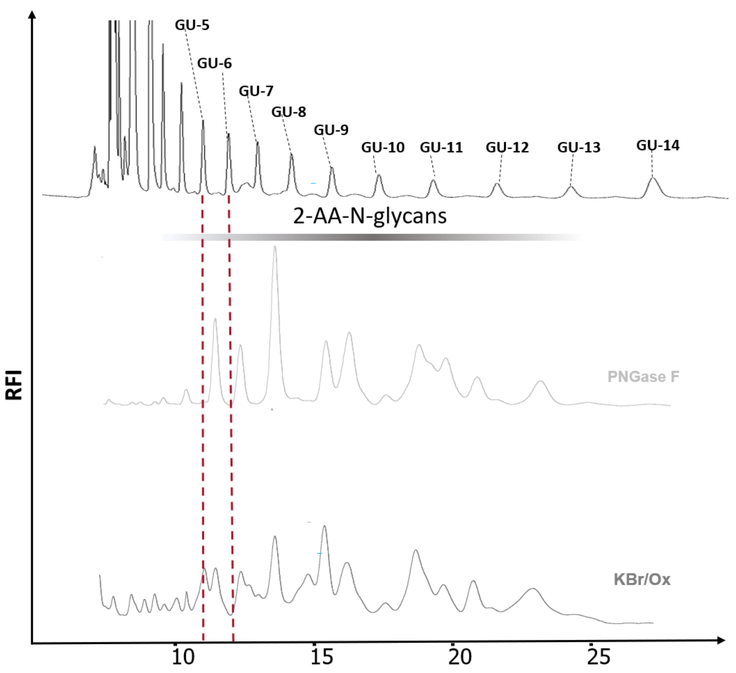
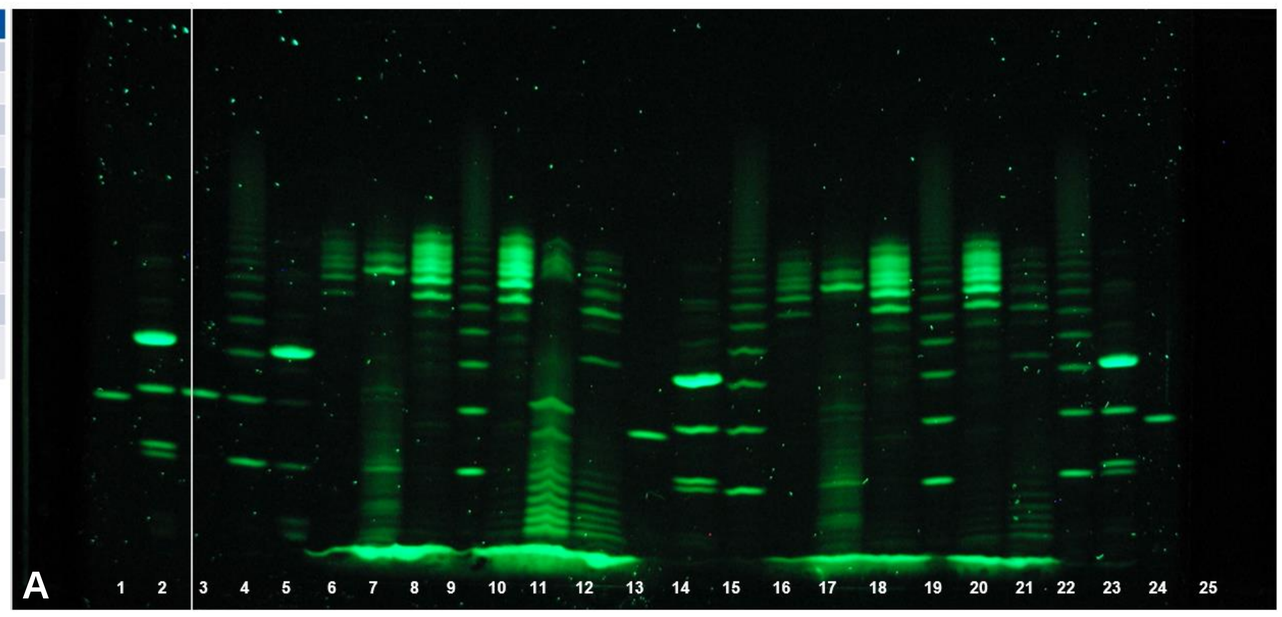


Fig. 4: Synoptic view of HILIC-FLD profiles of anthranilate labelled N-glycans from chicken ovalbumin. The pattern of ovalbumin N-glycans released by PNGase F matches the pattern released by Oxone® and bromide.

BHZ-FACE: SERVA HPE™ GlycoGel

ANTS-Samples in 0,1% SDS	Lane
Lactose	1 – 13 - 24
Mix: Mannose/Maltose/Maltotriose	2 – 14 - 23
Maltose	3 -
Partially Hydrolzed Dextran	4 – 9 – 15 – 19 - 22
Maltotriose	5 -
Ovalbumin N-Glycans	6 – 8 – 10 – 16 – 18 - 20
IgG1 N-Glycans	7 - 17
Etanercept – N-Glycans	11
Alpha1 anti-trypsin N-Glycans	12 - 21
Loading dye alone(Thorin I, not visible under UV light)	25

Electrophoresis System:
HPE™ BlueHorizon™ Flatbed Chamber
BluePower 3000 HPE™ Power Supply
HPE™ Chiller
Gel Dimensions: 125 x 250 mm x 0,43 mm
Elektrode Buffer: 1x SERVA GlycoBuffer (40 ml/wick)
Gel Type: SERVA HPE™ GlycoGel
Runtime and Power Settings:
U1 = 250 V; I1 = 25 mA; t = 30 min
U2 = 600V; I2 = 40 mA; t = 5 min
U3 = 800V; I3 = 50 mA; t = 50 min; 5° C
Detektion: UV 365 nm/ Filter 525nm



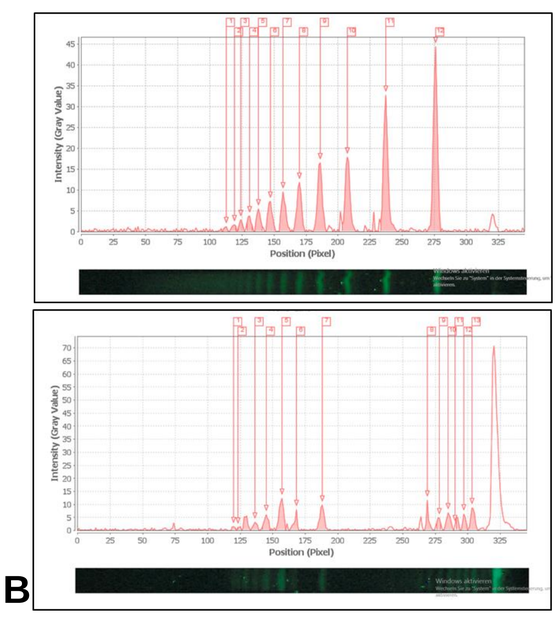


Fig. 5: A: Simultaneous and synoptic BHZ-FACE glycan analysis of 24 ANTS-labelled glycan samples in a single run. Note the ability of the system to remove excess ANTS from several different glycan samples. The number of samples that can be processed and analyzed in parallel is currently restricted only by the chosen mode of recording the UV illuminated gel. Due to its uniquely efficient technical setup and powerful heat dissipation by the flatbed chilling unit, BHZ-FACE separates 25 samples in parallel in one single run with the potential to upscale the throughput. B: Exemplary quantitative assessment of the glycan marker bands from the LabImage 1D electropherogram obtained for lane 15 and the AAT glycan sample bands (lane 12) (LabImage 1D Software, Kapelan BioImaging Solutions, Leipzig, Germany)

Perspectives: Chemical glycan release by hypobromite generated in-situ from Oxone® and bromide is a robust and cost efficient method to prepare glycan samples from glycoproteins and reduce cost-per-measure point. Ultrahigh resolution BHZ-FACE is extremely robust and reliable and yet capable of rapidly analyzing multiple fluorescently labelled glycan samples in a single run without the need for prior time- and loss-intensive label removal steps. Unlike any other existing glycan analysis technique, BHZ-FACE enables a direct synoptic within-run comparison between multiple different glycan samples.

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DFG Deutsche Forschungsgemeinschaft German Research Foundation
EFRE EUROPÄISCHE UNION Europäischer Fonds für regionale Entwicklung Investition in Ihre Zukunft

Literature

[1] Song X, Ju H, Lasanajak Y, Kudelka MR, Smith DF, Cummings RD. Oxidative release of natural glycans for functional glycomics. Nat Methods. 2016 Jun;13(6):528-34.