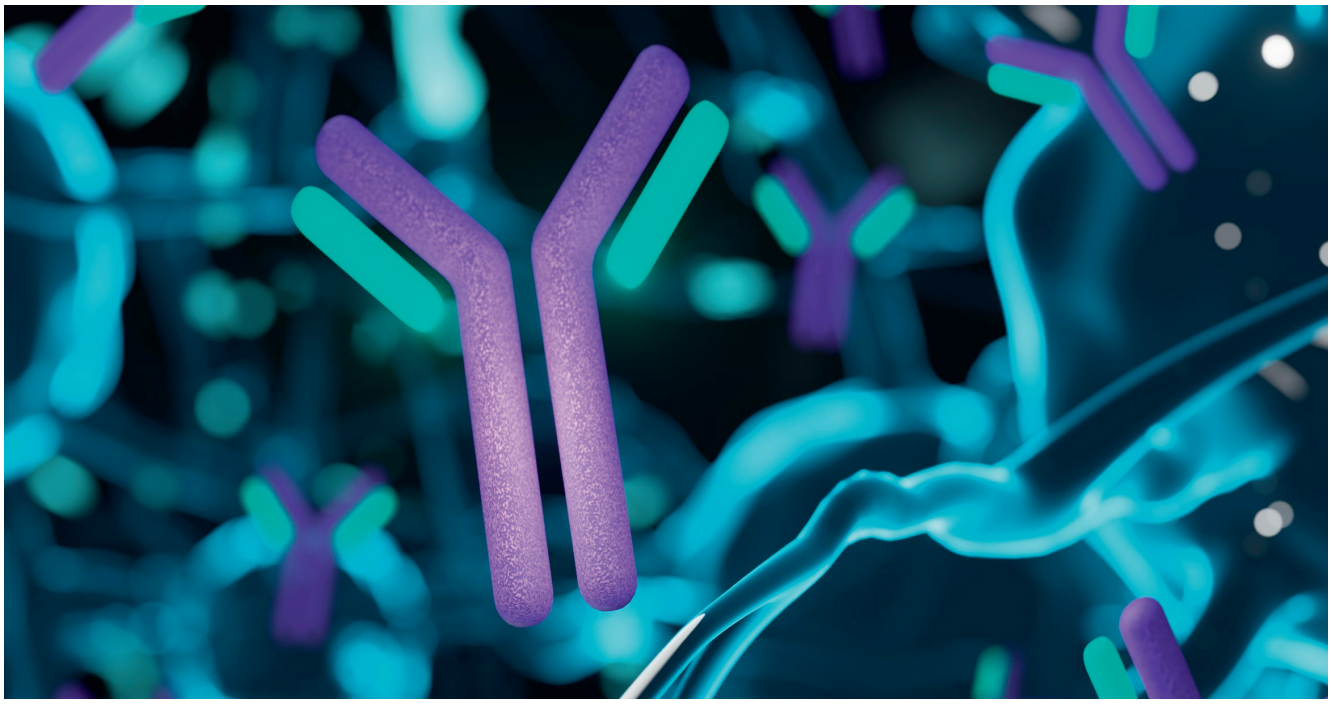
APPLICATION NOTE

Native AEX-MS analysis of IgG4-based mAbs

Cation exchange chromatography (CEX) is an excellent method of characterising the charge heterogeneity of biomolecules. This is also true for most commercially available monoclonal antibodies (mAbs). They are often IgG1-based and possess a high isoelectric point (pI) of usually ≥8. Therefore, CEX coupled with mass spectrometry (MS) is the traditional approach [1]. In contrast, anion exchange chromatography (AEX) has only been used for relatively acidic proteins.

However, for IgG4-based mAbs AEX may be an alternative approach. They possess a pI<8 and therefore CEX is less suitable. In addition, IgG4-based mAbs are becoming increasingly important. Already four IgG4-based mAbs are FDA-approved and at least six more candidates are in development [2].



This application note describes how the charge heterogeneity of IgG4-based mAbs with a pI between 6.1 to 7.3 (proprietary mAbs) can be successfully evaluated using a strong anion exchange (SAX) column, BioPro IEX QF, coupled to MS detection [3].

Table 1: Chromatographic conditions [3].

Column:	BioPro IEX QF (5µm) 100 x 4.6 mm ID
Part No.:	QF00S05-1046WP
Eluent:	A) 10mM ammonium acetate, pH 6.7
	B) 300 mM ammonium acetate, pH 6.8
Gradient:	0%B (0–2 min), 0–100%B (2–18 min), 100%B (18–22 min)
Flow rate:	0.4 mL/min
Temperature:	45 °C
Injection:	5 µg mAb sample
Detection:	NSI-MS (nanoelectrospray ionisation)
	UV
Sample:	6 In-house IgG4-based mAb, pI=6.1 – 7.3 (Regeneron)
Post column setup:	Post column stainless-steel tee to direct the majority to the UV detector
	Remaining sub-microlitre per minute flow directed to the NSI-MS

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The combination of AEX and MS requires a special setup (see Figure 1) in which a stainless-steel T-piece after the column divides the flow. Most of the flow is directed to the UV detector, while the remaining sub-microlitre per minute flow is directed to the nanoelectrospray ionisation mass spectrometer (NSI-MS). NSI is used because it can tolerate high salt concentrations of up to 600 mM ammonium acetate.

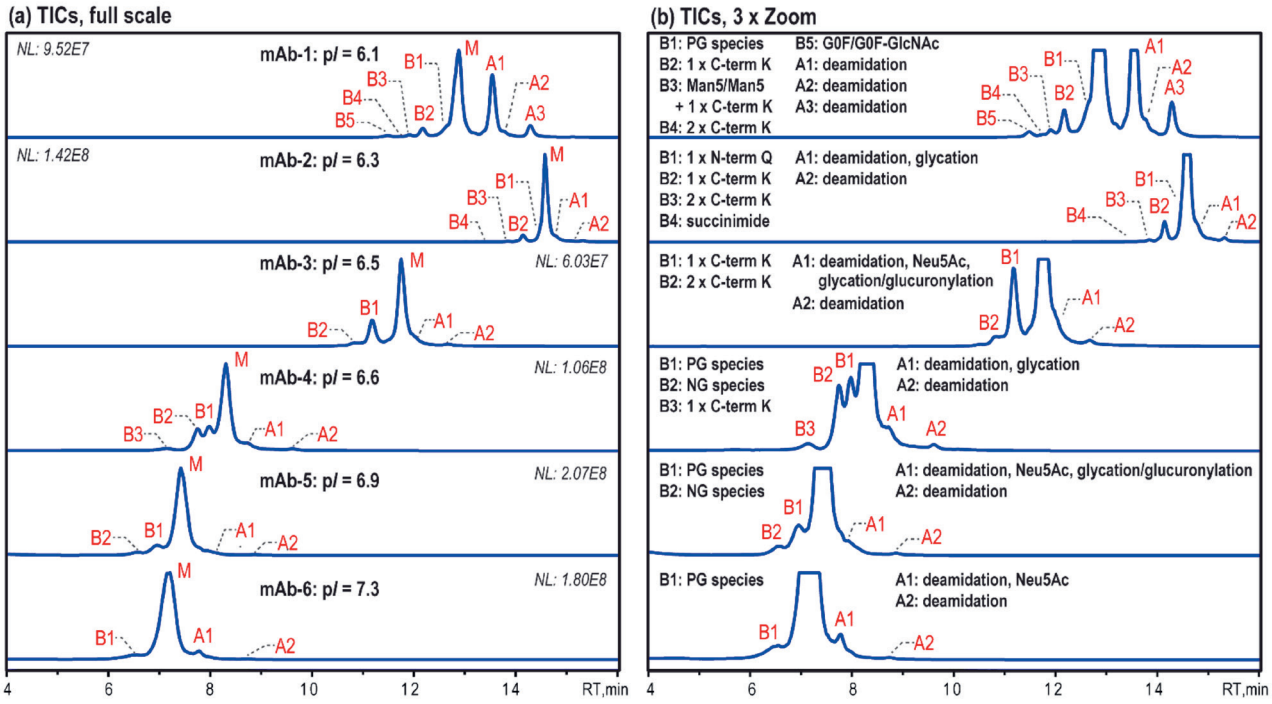

Figure 1: Native AEX-MS charge variant analysis of six different IgG4-based mAbs, shown basic (B) and acidic (A) variant peaks as well as the main species (M) [3].

Figure 1 shows that the AEX-MS method is suitable for IgG4-based mAbs with a moderate pI. The separation further improves as the pI gets lower.

mAb-6 has a pI of 7.3, which is higher than the mobile phase pH, but sufficient separation still occurs. This suggests that it is the surface charge rather than the intrinsic charge that causes the AEX-based separation. The acidic variants separated correlate with those commonly observed by CEX-MS such as deamidation, glycation, glucuronylation and sialic acid (Neu5Ac)-containing species.

Deamidated variants were found in several peaks. The additional abundant peak A1 of mAb-1 contains deamidation that correlates with a known deamidation site in its complementary determining regions (CDR).

However, the other mAbs tested lack deamidation sites in their CDRs, so that these two acidic peaks are likely to be due to site-specific deamidation in the Fc region.

The basic variants observed were identified as unprocessed C-terminal Lys (C-term K), non-cyclised N-terminal glutamine (N-term Q), succinimide and mAb species with different numbers of Fc N-glycans. In addition, the glycoforms Man5/Man5 with unprocessed C-term K and G0F/G0F-GlcNac were observed for mAb-1. mAb-4 demonstrates that this AEX-MS method is very sensitive to the macroheterogeneity of Fc N-glycosylation. The fully glycosylated (FG) main species is separated from the partially glycosylated (PG) peak B1 and the non-glycosylated (NG) species B2, which elute earlier.

Literature

[1] Y. Yan, A. P. Liu, S. Wang, T. J. Daly, N. Li, Ultrasensitive Characterization of Charge Heterogeneity of Therapeutic Monoclonal Antibodies Using Strong Cation Exchange Chromatography Coupled to Native Mass Spectrometry, Anal. Chem. 2018, 90, 13013-20.

[2] Kaplon, H.; Reichert, J. M. MAbs 2021, 13, No. 1860476.

[3] A. P. Liu, Y. Yan, S. Wang, N. Li, Coupling Anion Exchange Chromatography with Native Mass Spectrometry for Charge Heterogeneity Characterization of Monoclonal Antibodies, Anal. Chem. 2022, 94, 6355–62.