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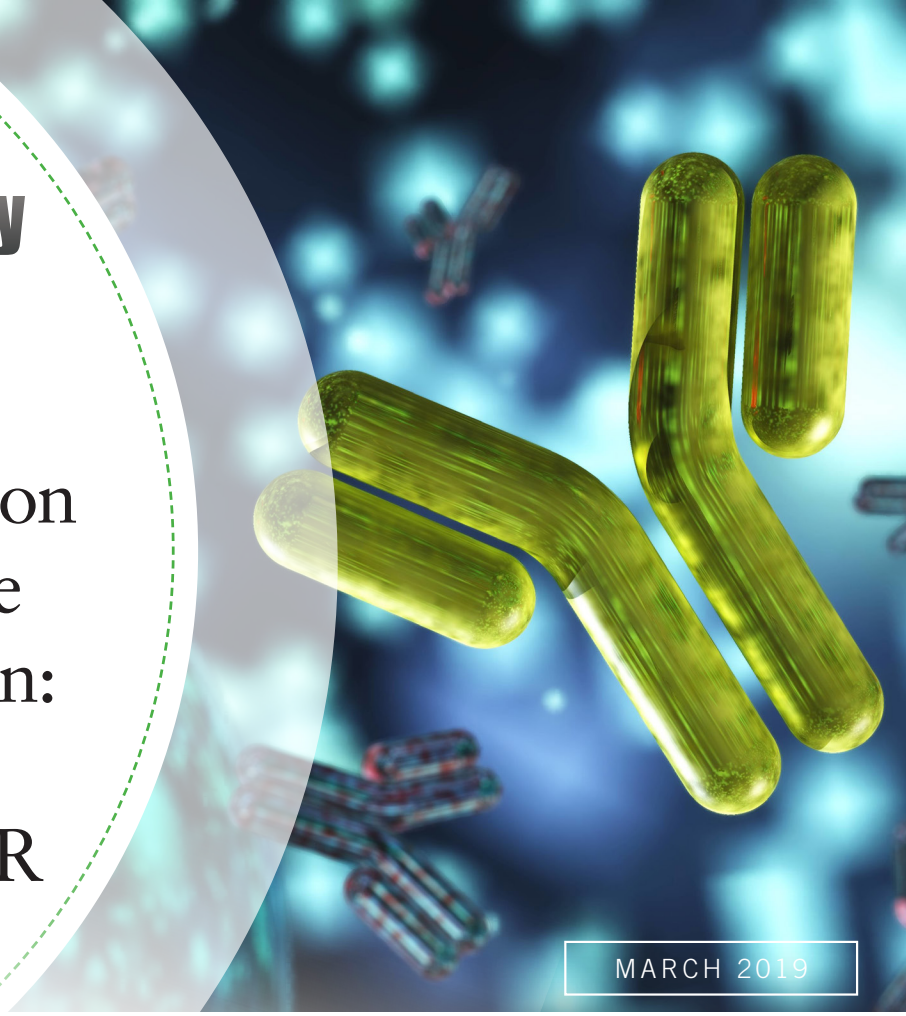

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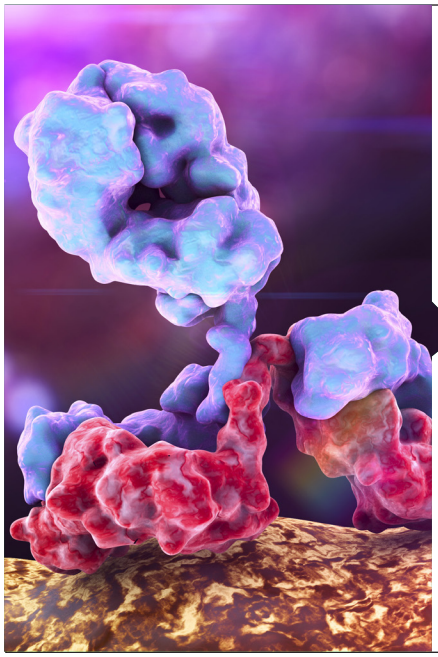

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Hydrophobic Interaction Chromatography of mAbs and ADCs: Not Just Another Way of Performing Reversed Phase-Like Separations

Hydrophobic interaction chromatography separates protein molecules based on hydrophobicity using non-denaturing mobile phases.

Andrew Coffey

Overview

Hydrophobic interaction chromatography (HIC) is a relatively new tool in the separations toolkit. Although sharing many features with reversed-phase separations, there are several differences between the methods. Both enthalpic (i.e., hydrophobic/ hydrophilic interactions) and entropic (i.e., hydration shell) interactions are responsible for retention. Conditions for the separation start under high salt concentrations leading to retention, with elution by reducing the salt concentration. HIC can provide relatively fast separations of antibodies and other proteins under gentle enough conditions to prevent denaturation and irreversible adsorption.

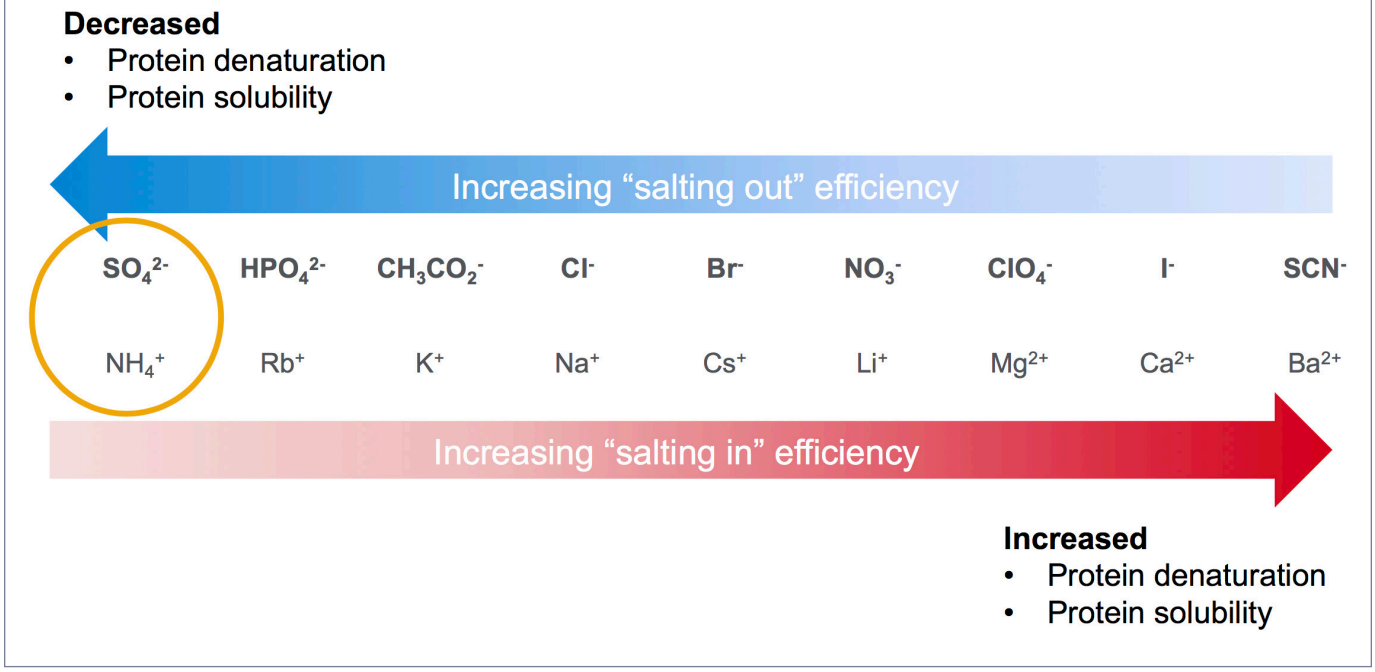
Introduction to HIC

One difference between HIC and reversed-phase chromatography (RPC) is the relatively polar stationary phase. While much RPC is performed with alkylbonded stationary phases, HIC phases often contain alkyl esters, ethers, and even amide groups. They are far less hydrophobic. Not surprisingly, the mobile phase in HIC is similarly much more polar to provide adequate interaction with the stationary phase.

Mobile phases in HIC typically contain very little or no organic solvent. Instead, mobile phase strength is controlled by the salt concentration. The phenomenon relates to the "salting out" often observed with proteins. Salts can screen the electrostatic and dipole–dipole interactions as well as disrupt the solvation shell of the molecules.

Figure 1 presents the effects of various salts on the stability and solubility of proteins in solution. Chaotropic salts appear on the right (e.g., $NaClO_4$) and will improve the protein's solubility, but tend

Figure 1: Understanding HIC mobile phase composition: Hofmeister series.



to disrupt the chains to the point where they unravel from their tertiary structure. Salts on the left, however, often stabilize the protein's structure, but increase the likelihood of adsorption and precipitation. Ammonium sulfate is the most commonly used salt for HIC because of its ability to induce adsorption onto the column.

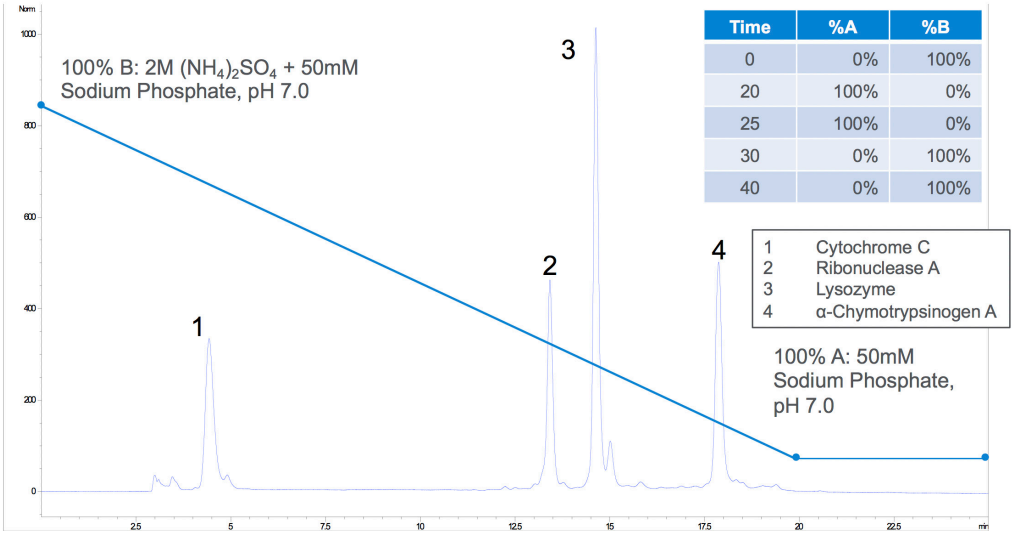
There is also an entropy-driven component to the separation. Water tends to form an ordered hydration layer around the protein as well as the stationary phase. These layers become disrupted and disordered when the protein approaches the stationary phase. The resultant increase in entropy makes retention favorable.

"HIC can provide relatively fast separations of antibodies and other proteins under gentle enough conditions to prevent denaturation and irreversible adsorption."

Figure 2 presents an HIC separation of cytochrome C, ribonuclease A, lysozyme, and alpha-chymotrypsinogen A. They are listed in order of least to most hydrophobic, which is, therefore, the order in which they are eluted.

Throughout the run, a 50 mM sodium phosphate buffer is used to provide a stable environment for the proteins,

Figure 2: HIC separation of standard proteins (4.6 x 100 mm column).



but ammonium sulfate salt is used as a gradient. The salt concentration is 2 M at the time of the injection, which creates a strong interaction with the stationary phase. As the salt concentration is reduced to 0% at 20 minutes, the solubility of the proteins in the mobile phase increases and they are eluted. While HIC can be run on most gradient LC systems, bioinert systems are best adapted for dealing with high salt mobile phases.

One challenge with HIC can be the danger of precipitating the sample onto the head of the column. The best way to avoid this problem is to make a concentrated sample solution and then dilute it with the concentrated ammonium sulfate. Bringing the sample matrix as close as possible to the initial mobile phase conditions will produce the best peak shapes and sensitivity. Doing so will also allow the operator to observe if precipitation is a problem before the sample is injected onto the column.

With careful column and experimental design, HIC can be characterized by very good repeatability, both injection to injection, and column to column. Like reversed phase (or any chromatography), good mass transfer is essential to having high efficiency and narrow peaks. To accommodate the large molecular size of the simple species, HIC columns tend to have much larger pore sizes, up to 450 angstroms or more. The column capacity is kept high by using fully porous particles.

The viscosity of the mobile phase presents some unique challenges. The slow mass transfer associated with large molecules in a viscous environment would make smaller particles the preferred choice, but the high viscosity puts pressure

limits on how small the particles can become. Most columns use 3.5 µm as the optimal compromise.

Because of the drastic changes in viscosity that result from the variation in salt concentrations, it is not recommended to rap-idly switch back to the initial mobile phase at the end of the gradient, as is typically done in RPC. Such a sharp change could damage the column. Rather, a relatively slow reverse gradient over several minutes should be used, followed by 2–3 column volumes to complete the re-equilibration.

Fortunately, HIC columns are relatively short. The most common choices are 3 cm and 10 cm. Limitations set by the higher viscosities are a contributing factor to this, but are not the only reason. HIC has a much higher selectivity for relatively small changes in the protein molecules than other separation techniques. As a consequence, it does not require the efficiencies (and related longer separation times) of lower selectivity separations.

One common approach when using high-viscosity mobile phases is to run at elevated temperature. Unfortunately, that is not a good solution for the separation of proteins. Minor increases in retention are seen, but more troublesome is the substantial degradation of the peak shape at even moderate temperature increases.

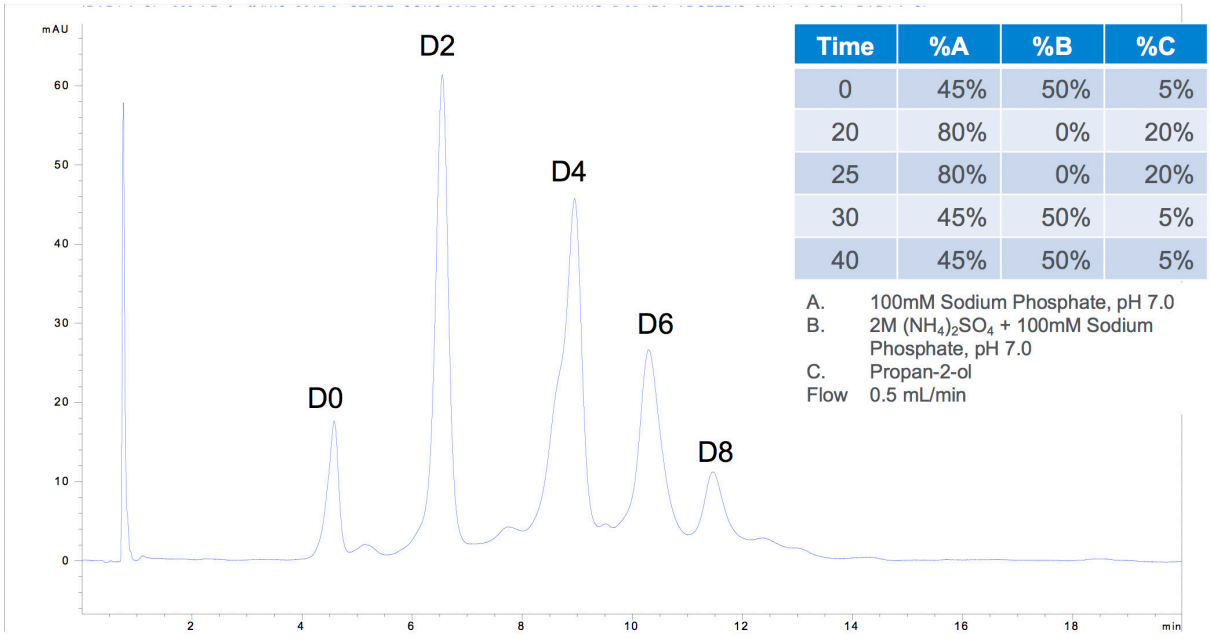
Applications of HIC

The ability of HIC to separate even closely related proteins makes it useful in the analysis of the purity of protein samples. In addition to contamination from outside sources, impurities may include modified proteins from the sample, including those that have been oxidized (particularly those containing methionine), de-amidated (asparagine transformed to aspartic acid), and rearranged (via the formation of isoaspartate). A glycosylated protein may be contaminated with non-glycosylated molecules. Antibodies may be contaminated by free light chains. Proteins that have been misfolded will also be separated. HIC can also easily identify the most common problem in protein production, which is the formation of stable dimers and higher aggregates. Deamidation presents particular challenges that even HIC may not be able to fully resolve. An antibody may contain as many as 50 asparagines that can be deamidated to aspartic acid. HIC can help determine if an antibody has been multiply deamidated, but it cannot distinguish which location within the molecule. Similarly, isomerization and oxidation can be detected, but which sites will not be readily identifiable. Additional analytical techniques will be needed to pinpoint these variants.

“The ability of HIC to separate even closely related proteins makes it useful in the analysis of the purity of protein samples.”

Interest in antibody-drug conjugates (ADCs) is growing, but these proteins can

Figure 3: Drug-to-antibody ratio (DAR).



Time	%A	%B	%C
0	45%	50%	5%
20	80%	0%	20%
25	80%	0%	20%
30	45%	50%	5%
40	45%	50%	5%

A. 100mM Sodium Phosphate, pH 7.0
B. 2M $(NH_4)_2SO_4$ + 100mM Sodium Phosphate, pH 7.0
C. Propan-2-ol
Flow 0.5 mL/min

be challenging to analyze. ADCs can be conjugated via the cysteines or the lysines of the antibody. Cysteine-linked ADCs tend to be much more fragile and prone to separation of the domains. In some cases, it may be more desirable to induce the domains to separate and analyze them independently, however HIC can be used without this need.

Figure 3 presents a separation of an ADC sample by HIC, showing resolution based on the number of drug molecules attached to each antibody. The greater the number of drug molecules attached, the more hydrophobic the conjugate hence the elution order is from low to high drug to antibody ratio (DAR). The presence of the drug makes an ADC much more hydrophobic than a mAb, requiring the addition of a small amount of organic solvent (in this case isopropanol) to the gradient to aid elution.

An obvious addition to the separation power of HIC is detection and identification using a mass spectrometer (MS). Unfortunately, the mobile phase used for HIC is not compatible, but there are two possible solutions. The first technique is fraction collection and desalting. The recent introduction of HIC columns with a higher hydrophobicity of the stationary phase allow lower salt concentrations to achieve the same retention, and may make the process of desalting easier downstream.

The second solution uses HIC as the first dimension in a two-dimensional separation. The eluent from the HIC column is injected via multiple heart cuts

onto a reversed-phase or size-exclusion column. The second separation uses a mobile phase that is compatible with mass spectrometry. One important consideration is that switching should be used to prevent the high salt components from coming out near the void of the second separation from entering the mass spectrometer.

Again, HIC often uses quite short columns. This becomes a consideration when designing a 2D separation. The separation time of the first dimension must be much longer than second dimension. The temporal resolution of the first dimension is set by the time it takes to perform a separation in the second dimension. This means that the RP or size-exclusion separation should be as short as possible, and that the HIC separation may require slow flow rates, and/or longer or multiple columns.

Conclusion

HIC is a powerful technique for the separation of peptides, proteins, and antibodies. The mobile phase is unusual in its particularly high salt concentrations and consequent high viscosity. The atypical nature of the mobile-phase chemistry makes for some unique challenges in terms of sample preparation, instrumentation, and interfacing with other techniques. Nonetheless, HIC has unparalleled selectivity for small changes in large molecules, thus allowing for separation in intact proteins based on minor modifications. It can be a particularly powerful technique for the evaluation and quantitation of antibody-drug conjugates. HIC is a useful addition to the relatively small number of techniques capable of nondestructively and selectively separating peptides and proteins while still in their native conformation.

“HIC has unparalleled selectivity for small changes in large molecules, thus allowing for separation in intact proteins based on minor modifications.”

Andrew Coffey
Senior Applications Chemist
Agilent Technologies

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Optimizing the Mobile Phase in SEC for Biopharmaceutical Applications

Buffer and salt concentrations influence separation quality in SEC, making their optimization critical when developing methods for biopharmaceutical applications.

Monoclonal antibody (mAb) products—such as mAbs themselves, antibody–drug conjugates (ADCs), Fc-fusion proteins, antibody fragments, and other therapeutic proteins—are used to treat a burgeoning array of medical conditions. Their highly complex manufacture and production involves multiple critical process steps, within which the avoidance of protein aggregation remains a major challenge.

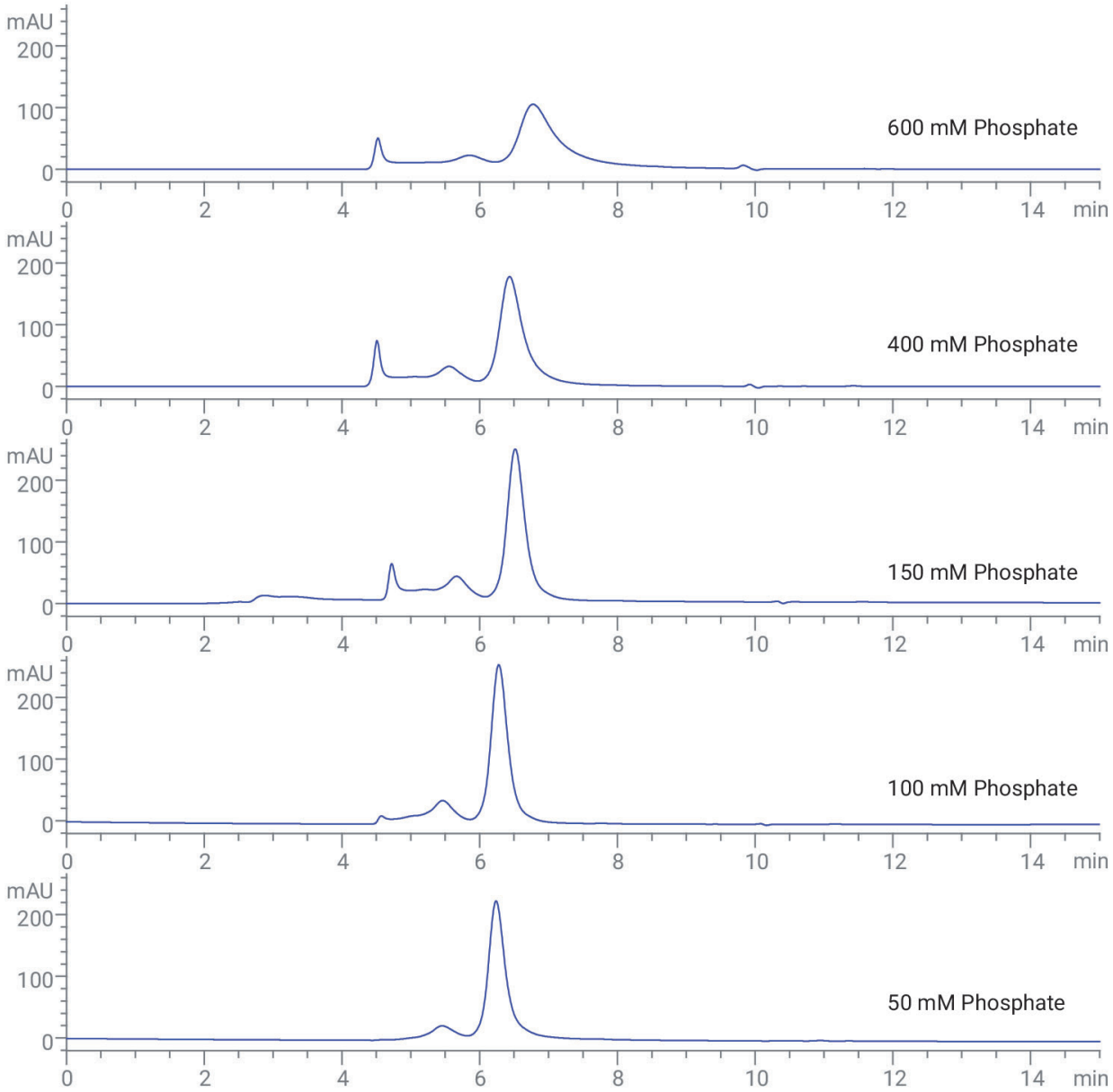
Protein aggregation has a negative effect on therapeutic product safety and efficacy and may lead to drug approval failures. Consequently, the amount of aggregation present in a protein or peptide is a critical quality attribute (CQA) that requires reliable and discriminating measurement in the biopharmaceutical industry. Size-exclusion chromatography (SEC) is considered the gold standard analytical method and is widely used for detecting monomers, dimers, aggregates and degradation products in therapeutic proteins and peptides.

When employing SEC for these applications, optimizing the mobile phase buffer and salt concentrations used with different chromatography columns is a key part of method development. Such optimization is essential to maximize separation quality and prevent secondary interactions between proteins and the column, which can cause peak tailing, peak shape distortion, and poor sample recovery and resolution. This article briefly describes optimization approaches and the effects of different mobile phase compositions on the chromatography of proteins of various sizes and chemistries.

Overall Approach

The experimental work reported on here was carried out using liquid chromatography

Figure 1: IgG separation at different phosphate concentrations.



instrumentation and AdvanceBio SEC columns (Agilent Technologies) to assess the effect of differing concentrations of phosphate buffer and sodium chloride on the chromatography of four biological materials: human IgG, an ADC mimic compound, insulin, and cytochrome C. Instrument conditions were set as: flow rate 1 mL/min, column temperature 25 °C; chiller 4 °C; and wavelength

Table 1: Tailing factors versus phosphate concentration.

Compound	MW (kDa)	pI	50	100	150	200	400	600
IgG	150	6.8	1.33	1.12	0.73	1.25	1.31	1.94
ADC	164	n/a	1.55	1.37	n/a	1.56	2.48	n/a
Insulin	5.7	5.3	2.48	2.36	2.44	2.51	1.73	1.09
Cytochrome c	12.3	9.3	2.1	n/a	1.7	1.3	1.10	1.06

Table 2: Monomer-dimer resolution versus phosphate concentration.

Compound	MW (kDa)	pI	50	100	150	200	400	600
IgG	150	6.8	1.33	1.18	1.41	1.31	1.14	0.79

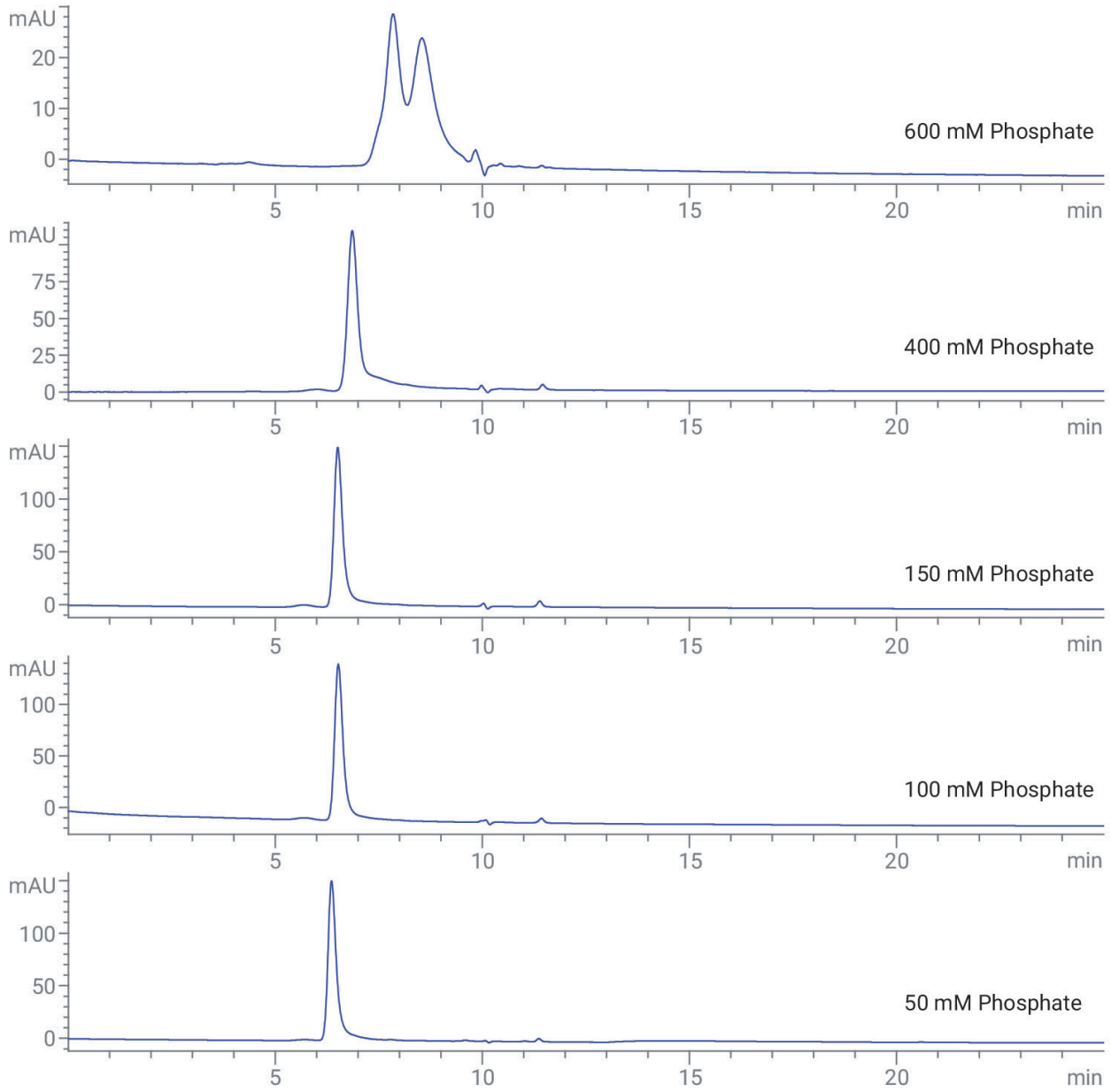
220 nm. A complete description of the experimental set up and reagents used is provided elsewhere.

Optimizing Phosphate Buffer Concentration

To examine the effect of phosphate buffer concentration, sodium phosphate solutions of 50, 100, 150, 200, 400 and 600 mM, at pH 7.0 with no sodium chloride, were used to run each of the four test samples using 10 µL injections of 1 mg/mL solutions.

The resulting chromatograms for human IgG presented in **Figure 1** show that phosphate buffer concentrations of 400 and 600 mM resulted in poor chromatography and that the IgG peak sharpened as buffer concentration decreased. The larger aggregate peaks at the two highest buffer concentrations suggested the presence of mobile phase-induced aggregation that would produce erroneous results. **Table 1** shows that tailing factors for the IgG monomer decreased with decreased buffer concentration down to 100 mM, while peak resolution between the IgG monomer and dimer increased with decreasing buffer concentration (**Table 2**). The lowest tailing factor and highest resolution were achieved at 150 mM, so this would be the recommended

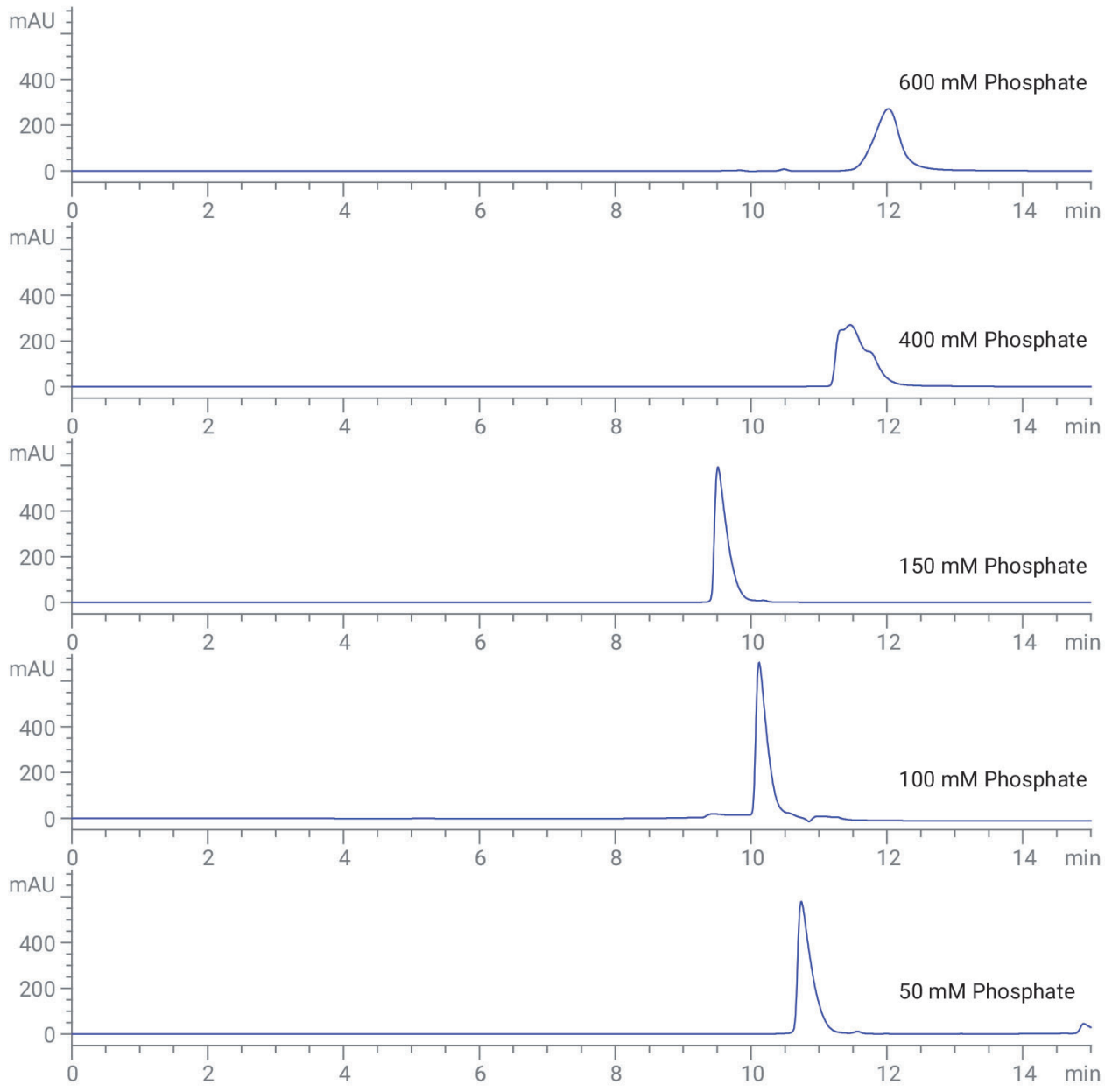
Figure 2: ADC chromatography at different phosphate concentrations.



phosphate buffer concentration for the IgG sample.

ADC mimic results are shown in **Figure 2**. At a buffer concentration of 600 mM, the ADC peak split, and was wide and tailing. Furthermore, there was a longer retention time as a result of secondary interactions (**Table 3**). Tailing factors decreased with decreasing phosphate concentrations, while peak shapes and sizes improved

Figure 3: Insulin chromatography at different phosphate concentrations.



(Table 1, Figure 2), making 150 to 50 mM the preferred concentration range for the buffer.

The known challenges associated with insulin SEC include problems with peak tailing and fronting. **Figure 3** shows the distorted peak shapes and poor recovery observed with insulin at 600 and 400 nM phosphate buffer, with taller, sharper peaks achieved at lower

Table 3: Retention times at different phosphate concentrations.

Compound	MW (kDa)	pI	50	100	150	200	400	600
IgG	150	6.8	6.24	6.28	6.29	7.55	6.44	6.77
ADC	164	n/a	6.36	6.52	n/a	6.51	6.96	n/a
Insulin	5.7	9.3	10.74	10.12	9.52	9.62	11.46	12.02
Cytochrome c	12.3	5.3	9.18	10.8	9.4	8.55	8.53	8.58

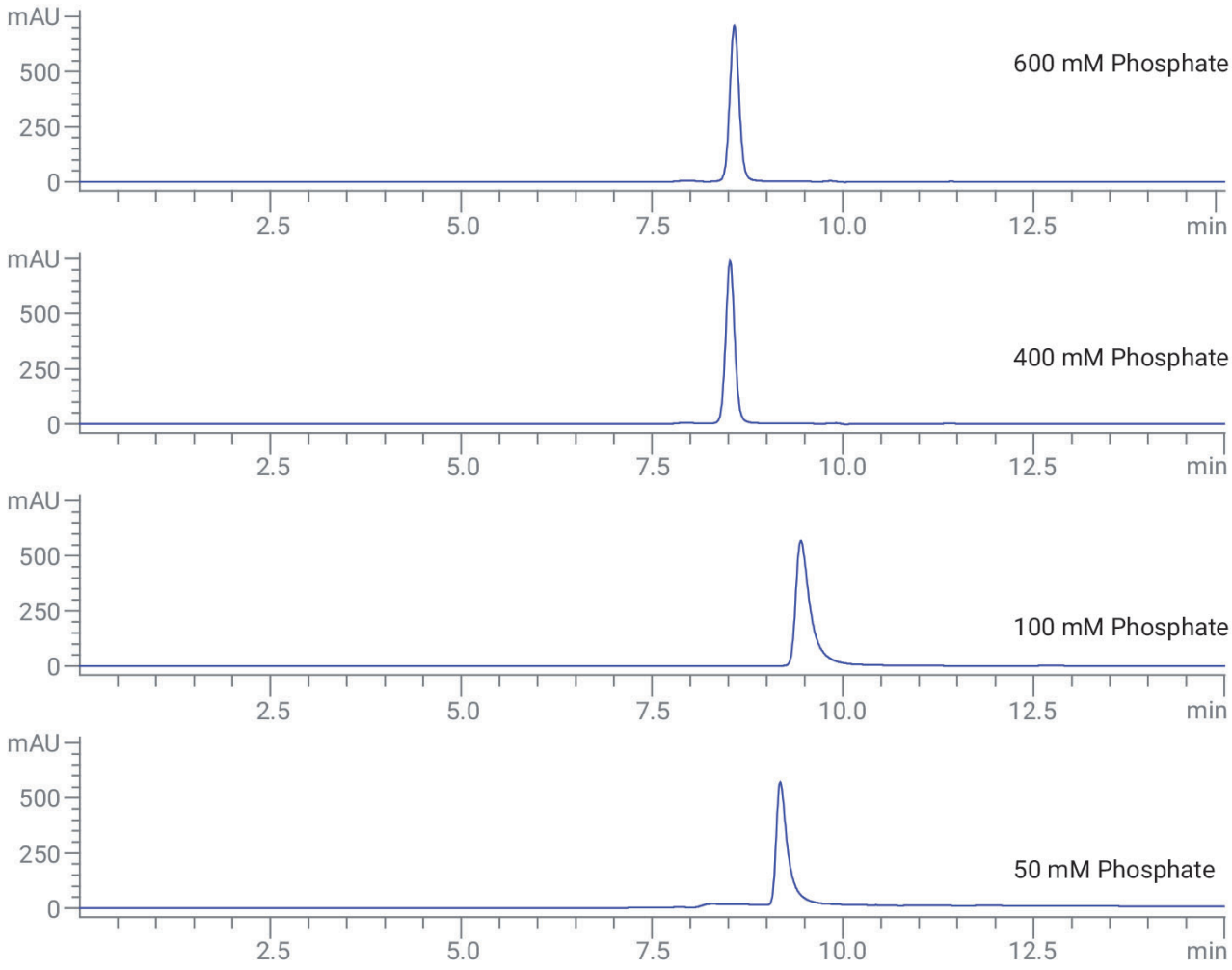
Table 4: Tailing factors versus 150 mM phosphate buffer and varying NaCl concentrations.

Compound	MW (kDa)	pI	0	50	100	150	200	500
IgG	150	6.8	0.73	1.16	1.19	1.19	1.21	1.62
ADC	164	n/a	n/a	1.51	n/a	1.43	1.43	1.52
Insulin	5.7	5.3	2.55	2.61	2.55	2.53	2.39	2.18
Cytochrome c	12.3	9.3	1.7	3.32	1.27	1.11	1.07	1.02

Table 5: Retention times at 150 mM phosphate and different NaCl concentrations.

Compound	MW (kDa)	pI	0	50	100	150	200	500
IgG	150	6.8	6.77	6.27	6.31	6.28	6.29	6.32
ADC	164	n/a	n/a	7.56	6.51	n/a	6.55	6.64
Insulin	5.7	5.3	9.52	10.29	9.43	10.51	9.48	9.7
Cytochrome c	12.3	9.3	9.4	8.72	9.4	8.61	8.75	8.67

Figure 4: Cytochrome c chromatography at different phosphate buffer concentrations.



concentrations. Tailing factors were constant along the 50 mM to 150 mM range (Table 1), but shifts in retention time, which were indicative of secondary interactions, occurred at 50 mM and 100 mM (**Table 3**). 150 mM phosphate buffer is, therefore, the preferred mobile phase (**Table 5**).

In contrast to the other proteins tested, cytochrome C exhibited decreasing tailing factors, better peak shapes, and higher peak areas at the higher phosphate concentrations (**Figure 4**). This pointed to fewer secondary interactions at these concentrations and indicated the preferred use of 400 and 600 mM phosphate buffers as the mobile phase.

Figure 5: IgG, 150 mM phosphate buffer with differing NaCl concentrations.

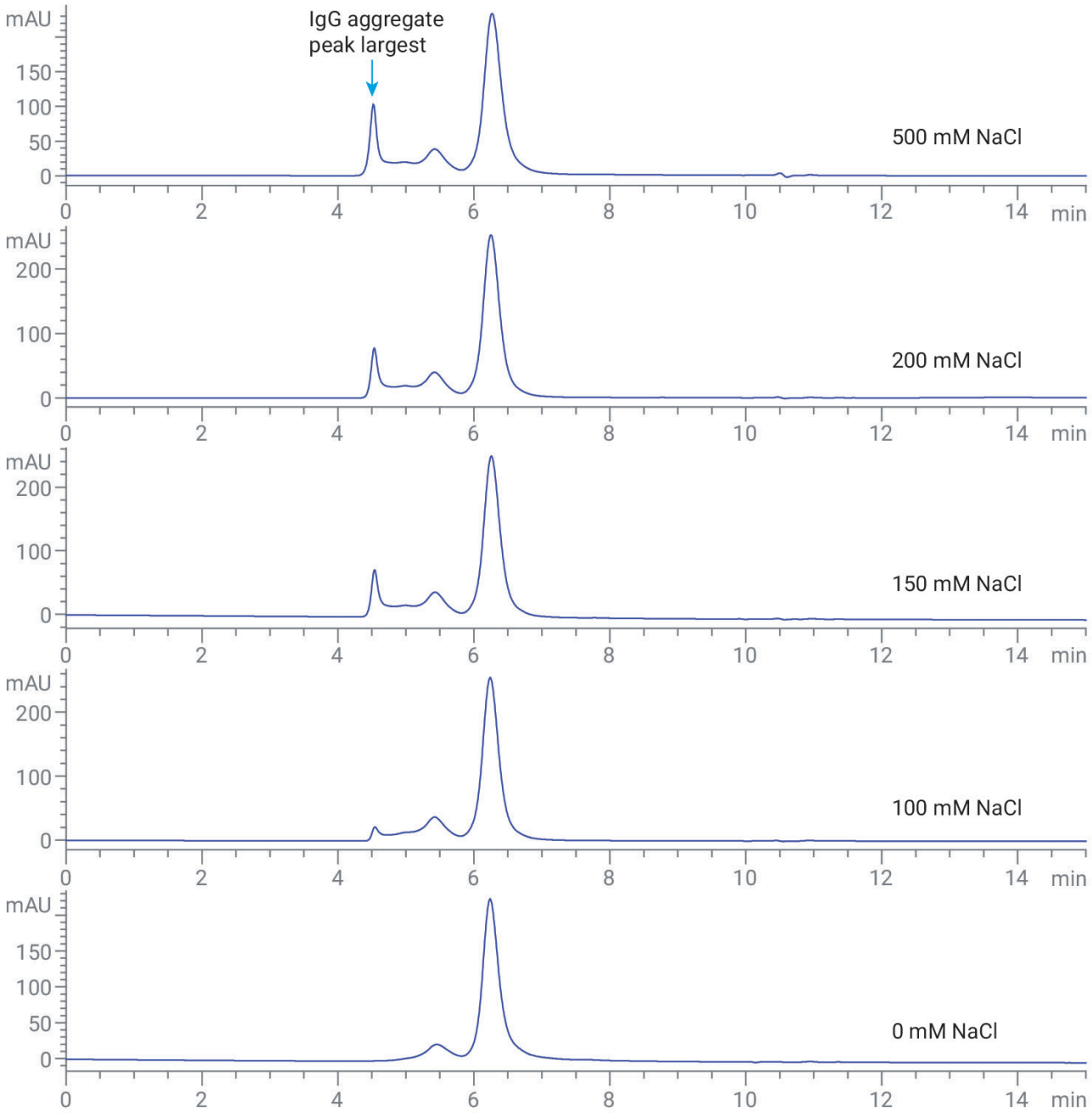
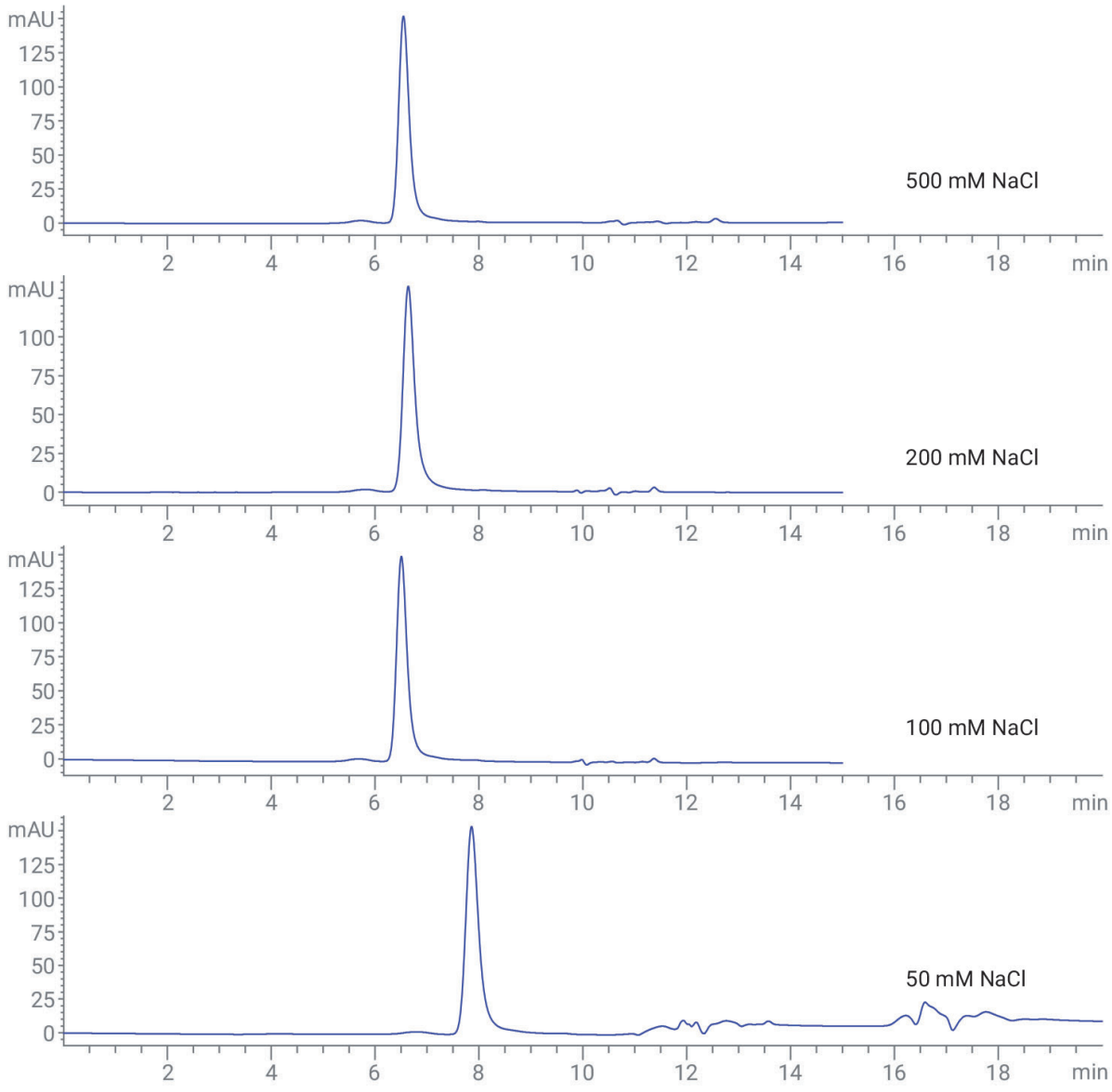


Figure 6: ADC, 150 mM phosphate buffer with differing NaCl concentrations.

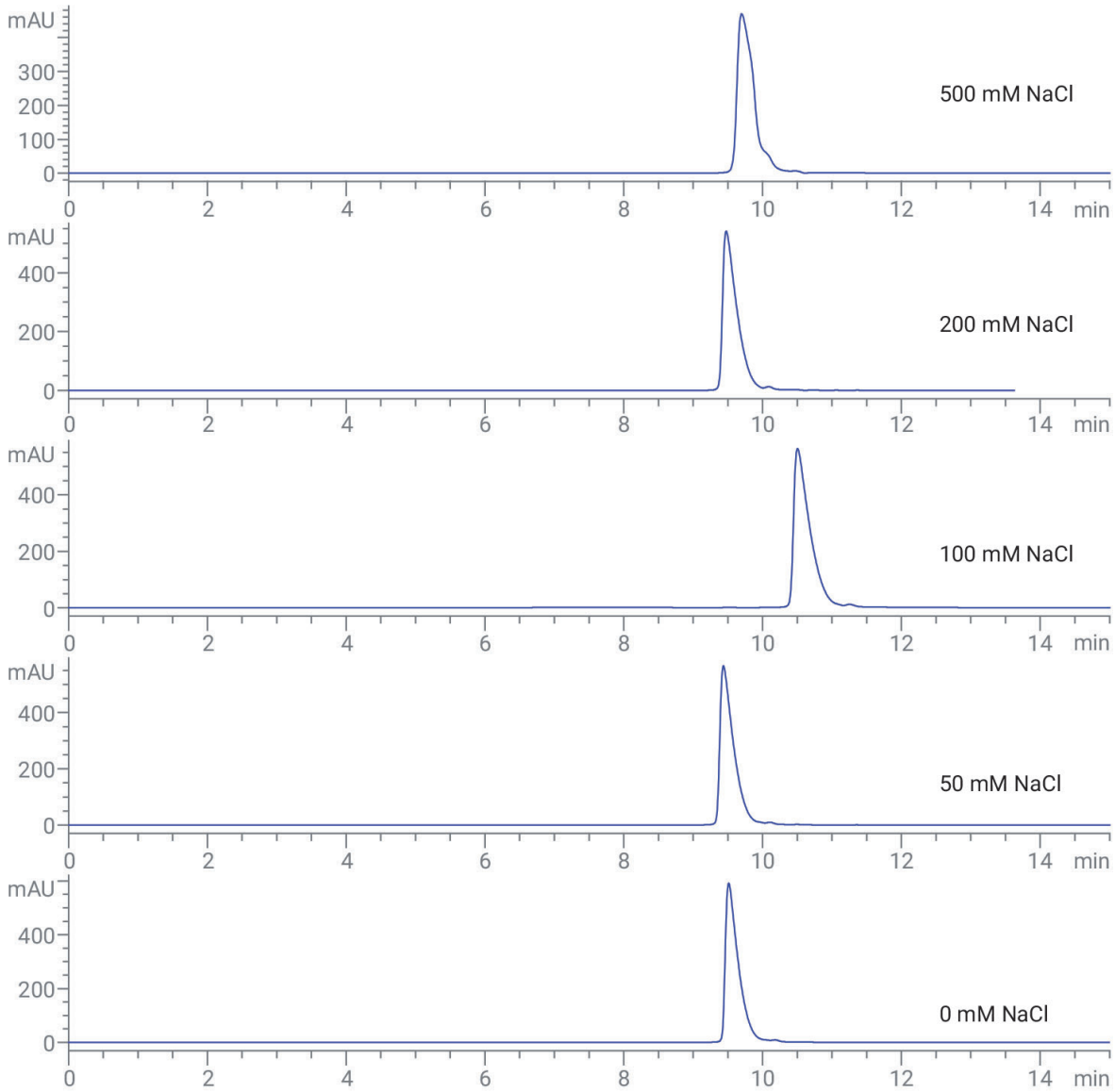


Adding Sodium Chloride to the Phosphate Buffer

Salt addition experiments on the same four sample materials were conducted using 150 mM phosphate buffer at pH 7.0, chosen as being a buffer that worked for most proteins. Sodium chloride was then added at concentrations of 50, 100, 150, 200 and 500 mM.

Figure 5 shows that for IgG, aggregates were present at every concentration of NaCl. In contrast, ADC mimic peaks were similar at each salt concentration (**Figure 6**) with the tailing factors remaining almost constant

Figure 7: Insulin, 150 mM phosphate with differing NaCl concentrations.

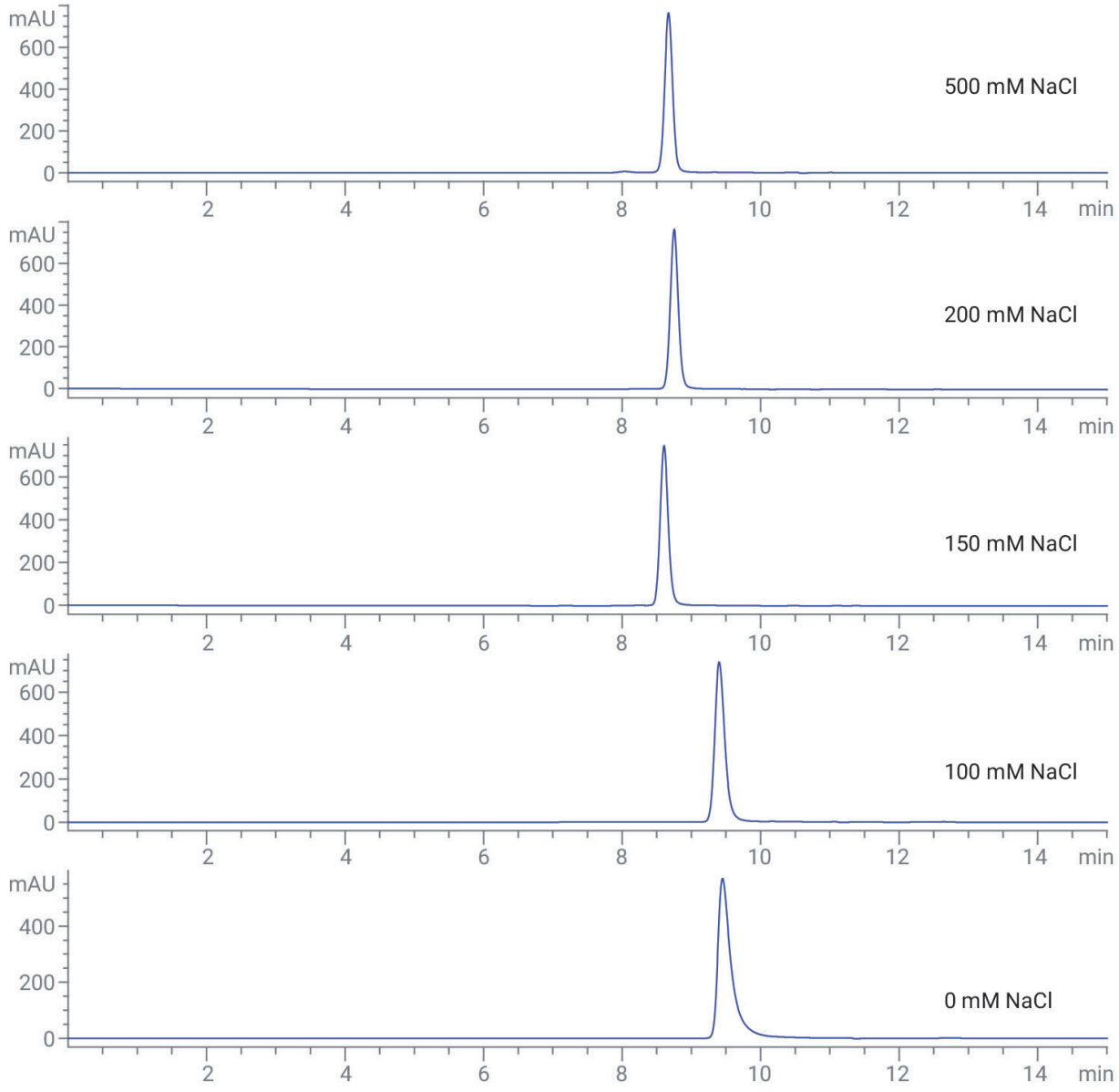


throughout (**Table 4**). This indicated that the effects of adding salt to the mobile phase were negligible.

The insulin peak was deformed at 500 mM salt, with better peak shapes from 200 mM to 50 mM (**Figure 7**). Since adding salt did not improve the peak shape, it was deemed unnecessary to do this when using 150 mM phosphate buffer.

Cytochrome C peak shape and size was similar from 500 mM to 100 mM NaCl (**Figure 8**). Tailing factors decreased with decreasing salt concentrations (**Table 4**)

Figure 8: Cytochrome c, 150 mM phosphate buffer with differing NaCl concentrations.



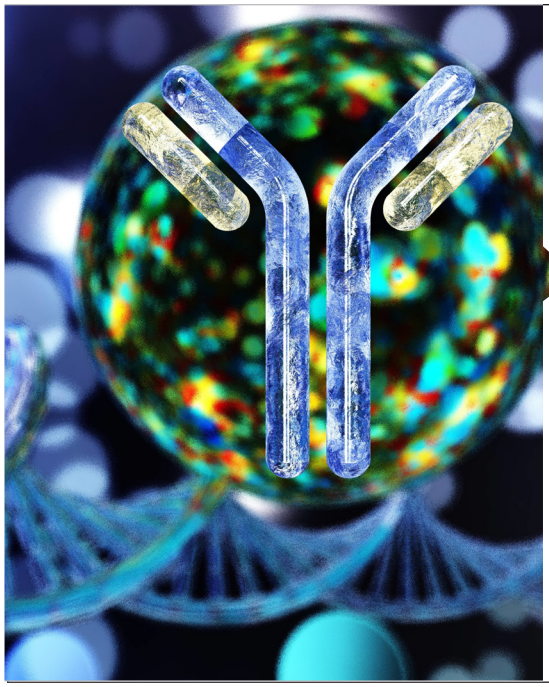
with retention times increasing from 100 mM down to 0 mM salt (**Table 5**). This all suggested that 150 mM phosphate buffer with 150 to 500 mM NaCl was the preferred mobile phase composition.

Conclusion

Mobile phase optimization is crucial to successful method development for protein SEC and is especially important when this analytical technique is used in critical applications. It is clear from the

work reported here that chromatographic separations can vary widely simply as a consequence of altering buffer and salt concentrations and highlights the importance of optimization. Achieving the accurate, reliable results essential to biopharmaceutical development and production depends on minimizing secondary interactions between proteins and the column, which can cause peak tailing, peak shape distortion, poor sample recovery and poor resolution. Mobile phase optimization also minimizes protein aggregation and reduces the risk of erroneous results. Buffers should additionally be selected to use the minimum possible volume and it is clear from the work here that salt should be added only if there is a demonstrable need. This contributes to reducing the risk of corrosion as well as wear and tear, which extends the life of the column.

"Mobile phase optimization is crucial to successful method development for protein SEC and is especially important when this analytical technique is used in critical applications."



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Improving Glycosylation Understanding and Monitoring in Therapeutic Proteins Through Faster Analysis

Approach to N-glycan preparation from therapeutic proteins speeds analysis and supports timely decision making on glycosylation.

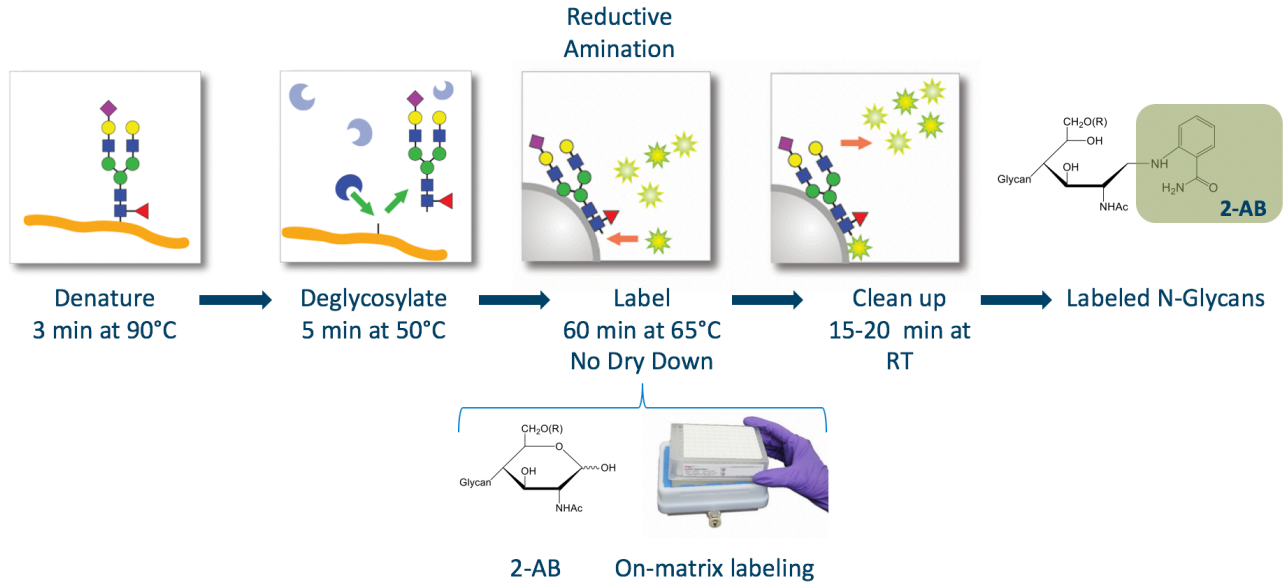
The glycosylation of human antibodies in nature plays a critical role in determining their structure and function. Similarly, glycosylation of recombinant monoclonal antibodies (mAbs) and Fc-fusion proteins for therapeutic use affects their pharmacokinetics, pharmacodynamics, and immunogenicity (1). Consequently, glycosylation is often a critical quality attribute (CQA) (2). Clear characterization and understanding of its impact are essential, as is close monitoring throughout the development and production process to accurately detect any changes. Historically, preparative methods involving the derivatization of glycans for analysis are lengthy, time-consuming and often unsuited to automated workflows, hampering rapid decision making. New approaches, such as the example presented here, are significantly reducing sample preparation time and supporting greater automation.

Approaches to N-Glycan Analysis

Analysis of N-glycans presents certain challenges because they are not inherently UV absorbing or fluorescent and ionize only poorly for mass spectrometry detection. As a result, it is usual to apply a fluorescent label, most commonly 2-aminobenzamide (2-AB) or 2-aminobenzoic acid (anthranilic acid; 2-AA), both of which modify the glycan by reductive amination (3).

Over the years, improvements in labeling technology have resulted in higher fluorescence and greater MS sensitivity. However, protocols for 2-AB labeling have tended to require multiple prolonged incubation periods. One goal is to avoid the risk of incomplete deglycosylation, resulting in overnight digestion becoming the norm. Reductive amination included a preceding step to dry the glycans before labelling and then reactions to affix the 2-AB label, which also needed lengthy

Figure 1: Gly-X 2-AB Express workflow for release and labeling of Nglycans



incubation. In addition, the older clean-up cartridges used to remove excess 2-AB reagent ahead of liquid chromatography (LC) often proved incompatible with high throughput or automated workflows.

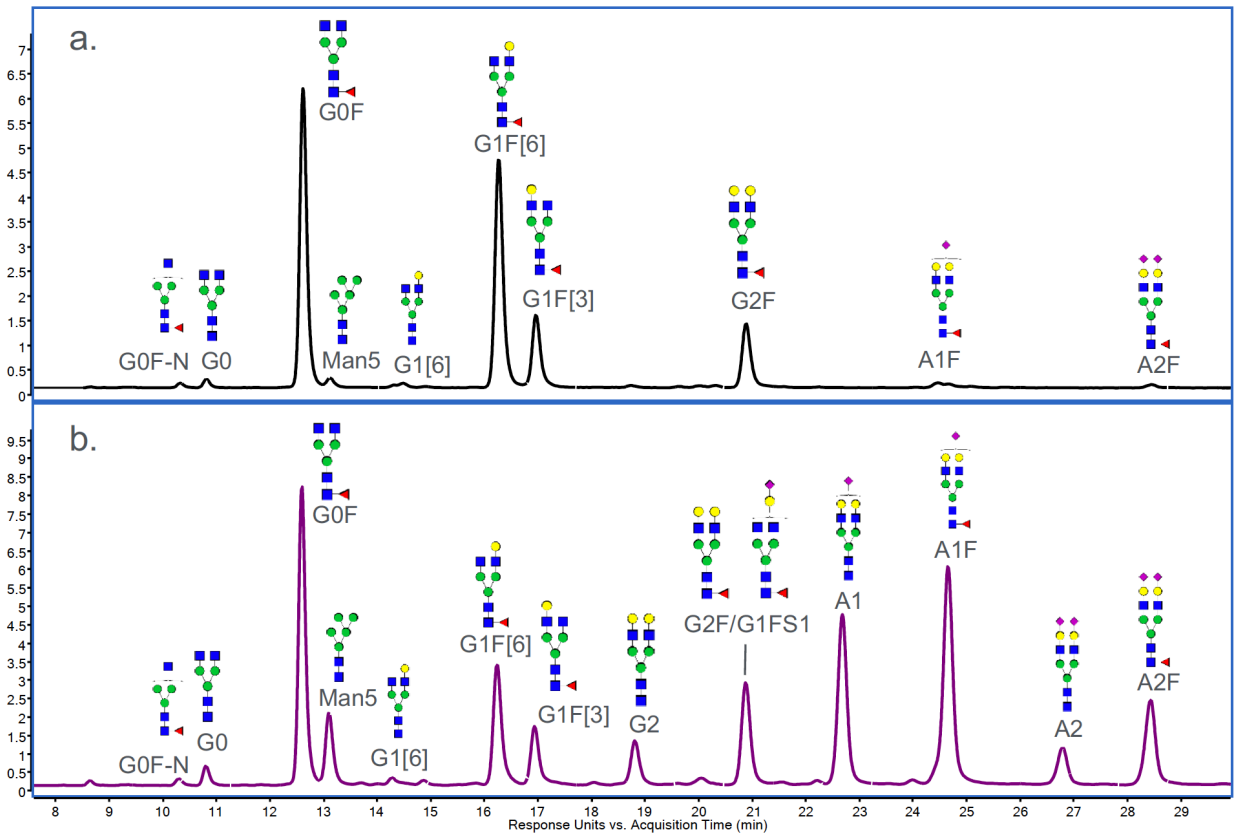
Work presented below illustrates the successful application of a rapid methodology (Agilent AdvanceBio Gly-X 2-AB Express Kit) in the preparation of released N-glycan samples from two therapeutic proteins for LC–fluorescence analysis.

> "Analysis of N-glycans presents certain challenges because they are not inherently UV absorbing or fluorescent and ionize only poorly for mass spectrometry detection."

Accelerating Sample Preparation

The Gly-X 2-AB kit used here includes all of the high-level steps for N-glycan sample preparation: denaturation, deglycosylation, labeling and sample clean up. To illustrate its application, the experimental protocol shown in **Figure 1** was used to prepare N-glycan samples from the therapeutic proteins MabThera and Enbrel. The resulting samples were

Figure 2: Representative chromatograms showing separation of 2-AB labeled N-glycans from a. MabThera and b. Enbrel.



analyzed using liquid chromatography with fluorescence detection (LC/FLD) and MassHunter Qualitative Analysis Software (Agilent). Full experimental details are available in Reference 4.

The first step in the workflow, denaturation of the target protein, involves the addition of denaturing agent to the sample followed by incubation at 90°C for three minutes. Effective unfolding of the protein at this stage then allows for highly efficient in-solution cleavage of N-glycans using the enzyme PNGase F, requiring only five minutes incubation at 50°C (5). Labeling and clean up are conducted on a HILIC-based solid-phase stationary support where glycans, followed by the 2-AB labelling reagents, are loaded to the stationary phase and incubated for one hour at 65°C. Acetonitrile washes are then used to clear excess reagent, followed by water elution of the labeled glycans. A dry-down step is not required, and the entire process is complete in about two hours.

Table 1: LC method used to analyze N-glycans.

LC Method			
Column	Agilent AdvanceBio Glycan Mapping, 2.1 x 150 mm, 1.8 µm, p/n 859700-913		
Column Temp	40 °C		
Mobile Phase	A = 50 mM ammonium formate, pH 4.5 B = acetonitrile		
Flow Rate	0.5 mL/min		
Gradient Program	**Time (min)**	**% B**	**Flow Rate (mL/min)**
	0.0	82	0.4
	2.0	82	0.4
	2.5	77	0.4
	48.0	62	0.4
	49.0	40	0.4
	51.5	40	0.4
	52.0	82	0.4
	54.0	82	0.6
	58.0	82	0.6
	58.5	82	0.4
Injection volume	1 µL (equivalent to glycans from 0.4 µg protein)		
Detection	Agilent 1290 Infinity II FLD Excitation 260 nm Emission 430 nm		

Results and the Importance of Reproducibility

Representative chromatograms, showing the separation of 2-AB labelled glycans from MabThera and Enbrel after LC/FLD analysis, are illustrated in **Figure 2.** MabThera has the simpler glycosylation pattern, while Enbrel exhibits higher relative levels of sialylated glycans.

One of the key requirements of sample preparation is that it must be reproducible. When comparing production lots of the mAb, it is essential that any measured variability genuinely arises from changes in the sample and not from artifacts of sample handling or analysis. **Table 1** shows an LC method used to analyze three separate preparations (performed as described previously) of a MabThera sample. **Table 2** presents the relative percent area of the major glycan species detected and reports

Table 2: Relative % area of major N-glycan species from three preparations of a MabThera sample.

		Relative % Area					
Glycan	**RT**	**1**	**2**	**3**	**Average**	**Standard Deviation**	**%CV**
G0F-N	10.32	0.56	0.57	0.57	0.57	0.01	1.02
G0	10.81	1.09	1.01	0.98	1.03	0.06	5.54
G0F	12.62	39.85	39.31	39.33	39.50	0.31	0.78
Man5	13.12	0.74	0.62	0.69	0.68	0.06	8.82
G1[6]	14.47	0.66	0.6	0.62	0.63	0.03	4.88
G1F[6]	16.27	34.65	34.81	34.67	34.71	0.09	0.25
G1F[3]	16.96	10.65	10.47	10.6	10.57	0.09	0.88
G2F	20.89	10.15	10.78	10.83	10.59	0.38	3.58
A1F	24.45	1.13	1.26	1.17	1.19	0.07	5.61
A2F	28.44	0.52	0.57	0.54	0.54	0.03	4.63

the average percent area, together with standard deviation and relative standard deviation (%CV). With the exception of the lower abundance glycans, the variations between sample preparations are low. Since precision is more challenging at the limits of detection, higher variation is to be expected for these peaks.

Comparability with Other Methods

Any decision to change sample preparation methods is not trivial. New methods must deliver data that is superior, or at the very least equivalent, to that from existing methodologies while also offering significant practical advantages. A major factor in the continued use of 2-AB as a label, for example, is the ability to compare results with older data from other 2-AB labelling protocols. **Figure 3** provides an example of the good comparison achieved between samples prepared using this rapid new methodology and those of an earlier generation technique.

Figure 3: Samples produced using the Gly-X 2-AB Express kit produce data equivalent to samples prepared using older methods, such as GlykoPrep 2-AB shown here. n=3 for all data.

Conclusion

Glycosylation is a feature of many biotherapeutic proteins and, since glycan composition may directly affect the safety and efficacy of the product, is often a CQA. N-glycan analysis is therefore critical in the development and production of therapeutic proteins. New approaches to the preparation of samples for analysis are significantly reducing the time this takes—dropping from a full day down to around two hours—and therefore are accelerating the time to results. The work described here shows the speed, reproducibility, and comparability with previous methods of a new protocol (Agilent AdvanceBio Gly-X 2-AB Express Kit) for the release and preparation of N-glycan samples from mAbs, using 2-AB labelling, for LC/FLD analysis. The ability to produce more timely results enables faster, more-informed decision making, supporting the requirements of both the development and production environment in ensuring the safety and efficacy of therapeutic drugs.

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