

# How Useful is the Whole-Column- Imaging Capillary Isoelectric focusing (CIEF) in the Detection of Different Modifications of Monoclonal Antibodies

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# Objectives

- Is it suitable to capture the characteristic pattern changes in bioanalysis, formulation and stability studies for therapeutical monoclonal antibodies?
- Parameters to evaluate
  - Sensitivity
  - Speed

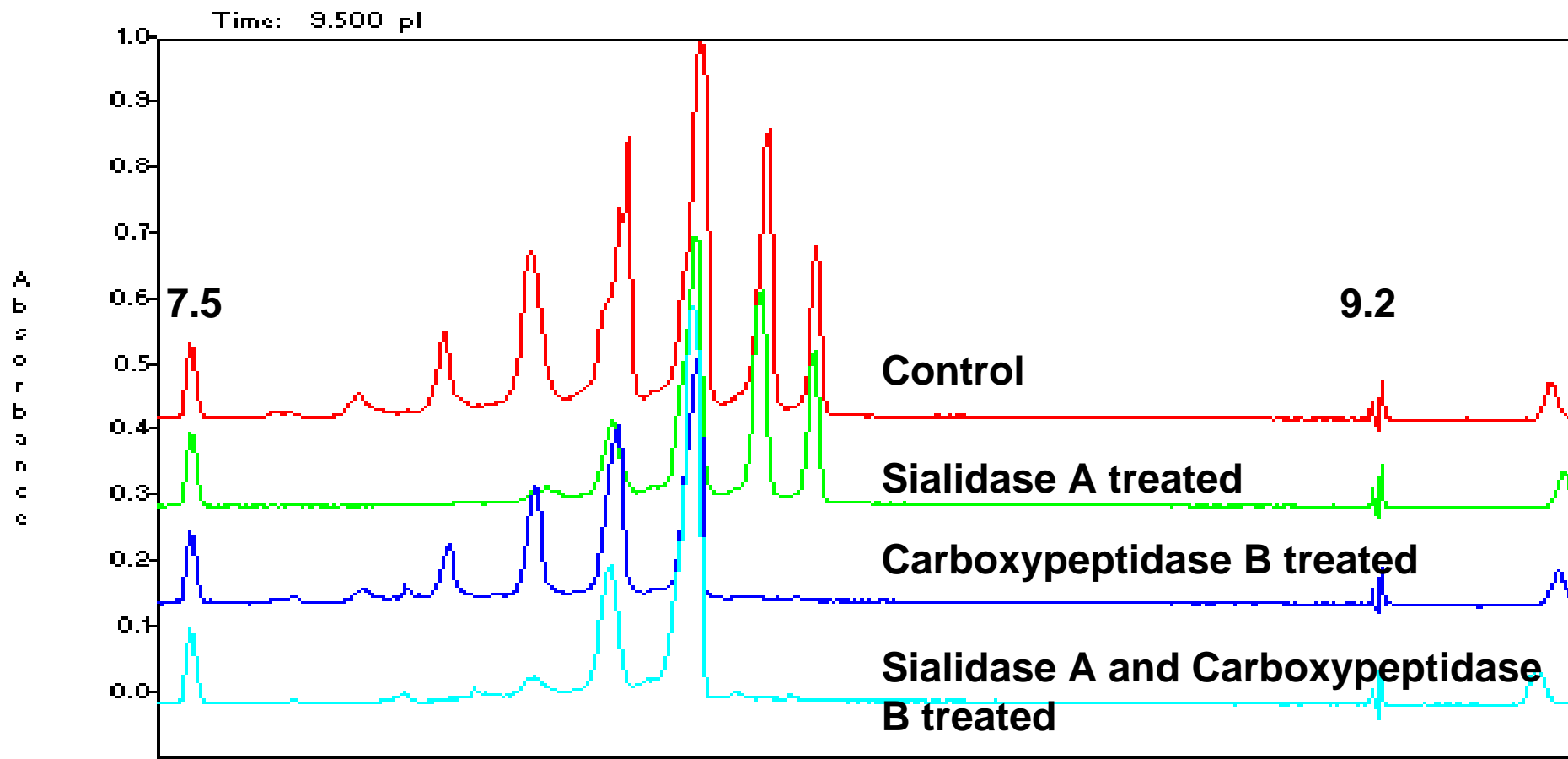
# Advantages of WCID-CIEF

- It does not require the mobilization step
- Therefore shortens the analysis time
- Avoid the possible distortion of pH gradient, resulting in more accurate pI value estimation of the interested protein.

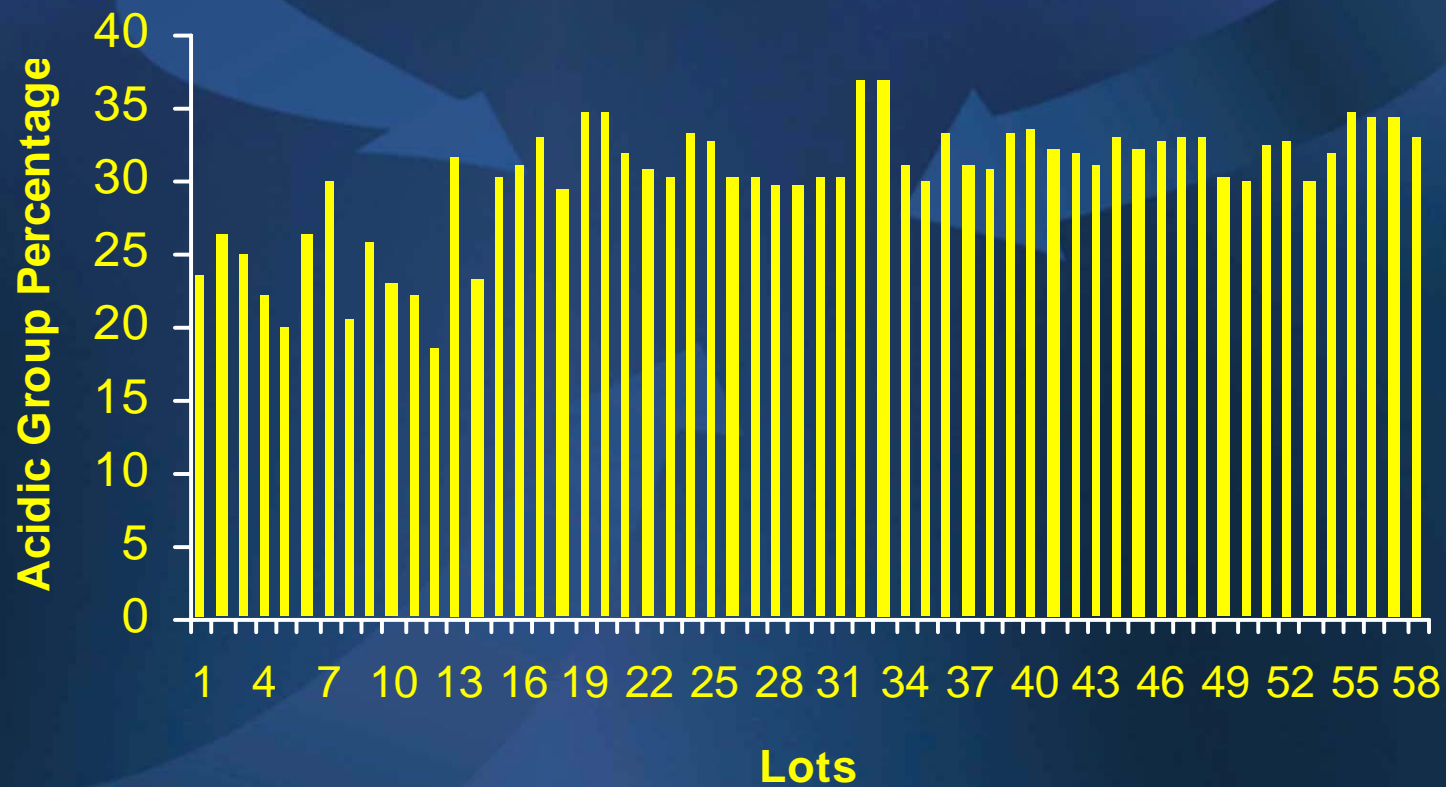
# Modifications and Manipulations Evaluated in this Study

- Sialylation
- C-Terminal Lysine Variation
- Deamidation
- Thermal Stability – Heat-Induced Modifications
- Light-Induced Modifications: Aggregation and Photo-Oxidation
- Oxidation:  $H_2O_2$
- Chemical Manipulations – Biotinylation and PEGylation

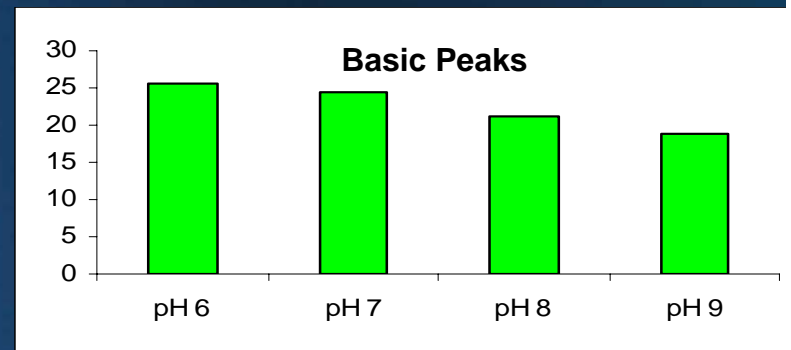
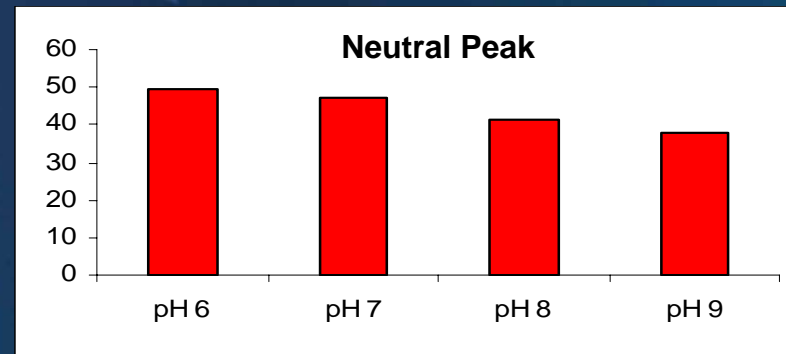
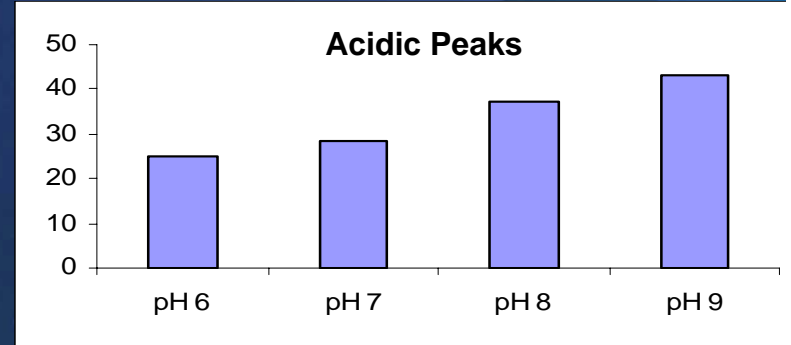
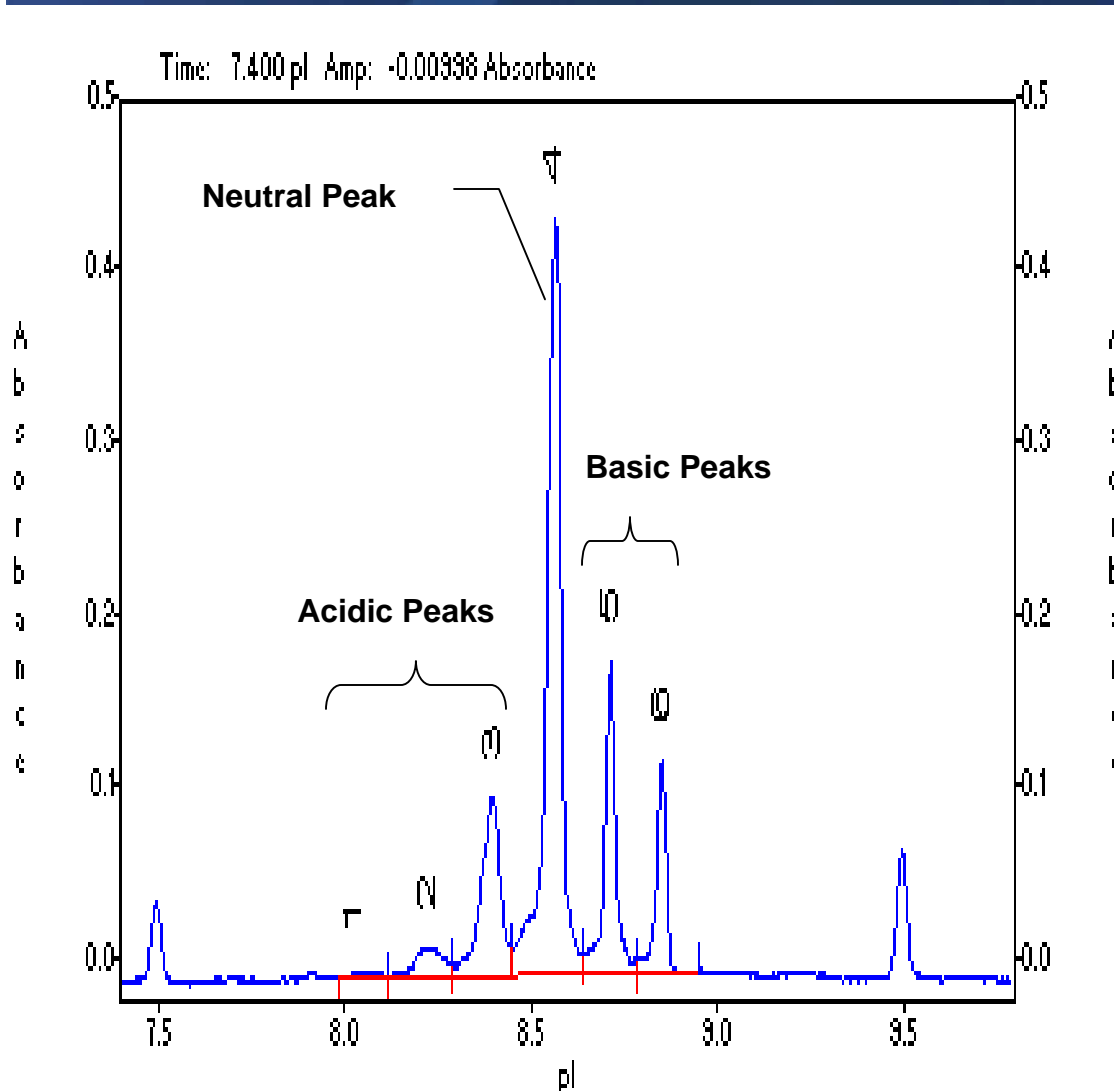
# Sialylation and Lysine Variations of MAb4



# Variation in Product Development

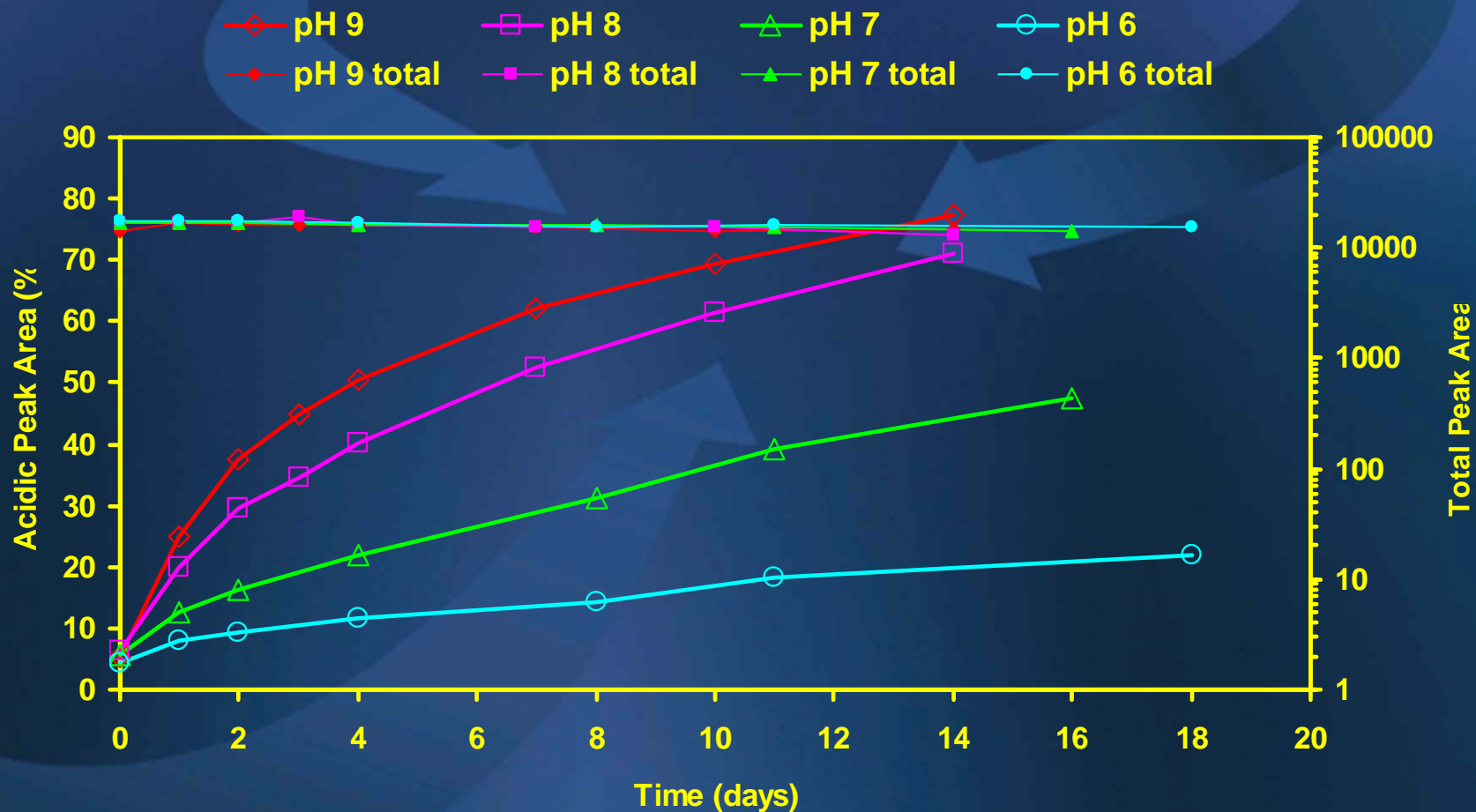


# Deamidation A Function of pH Values



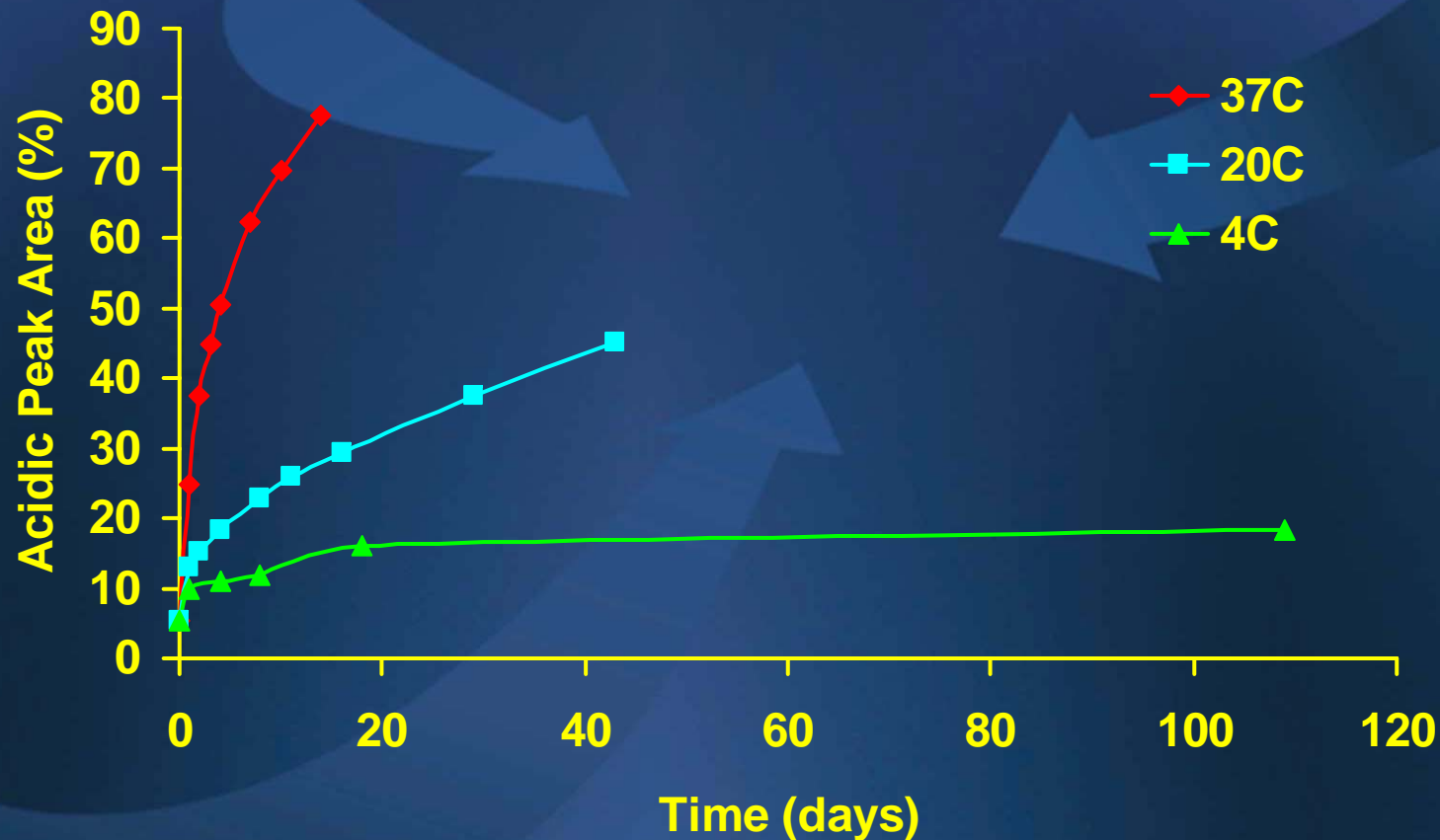
# Deamidation

## MAb4 Neutral Peak at Different pH



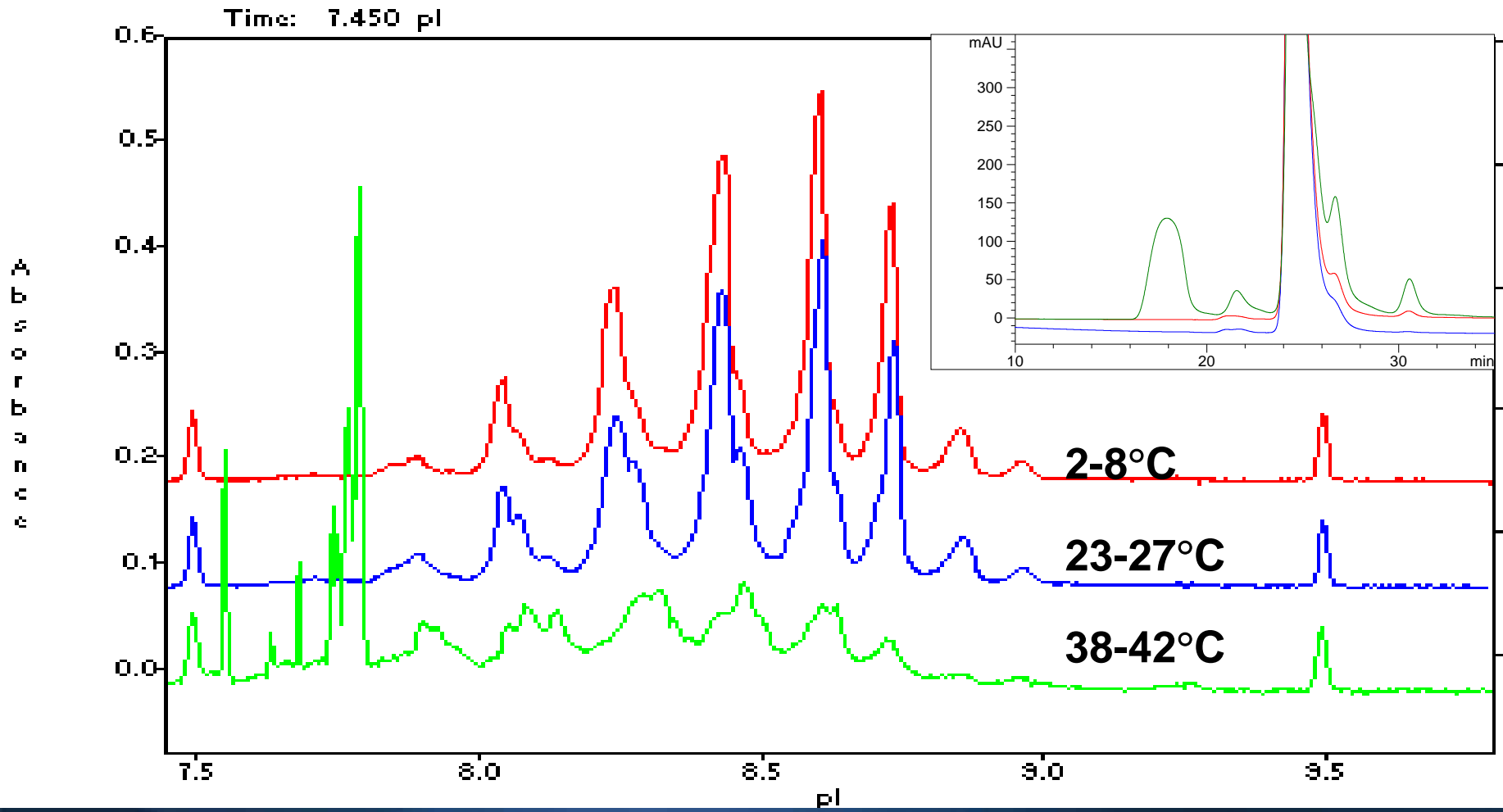
# Deamidation

## A function of temperature



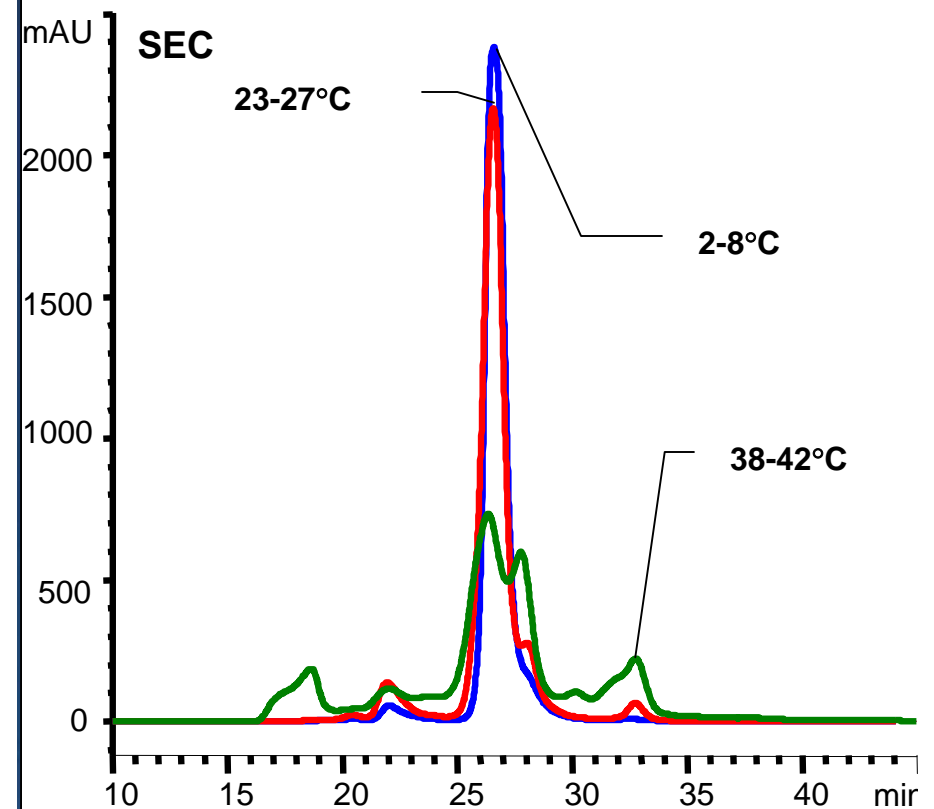
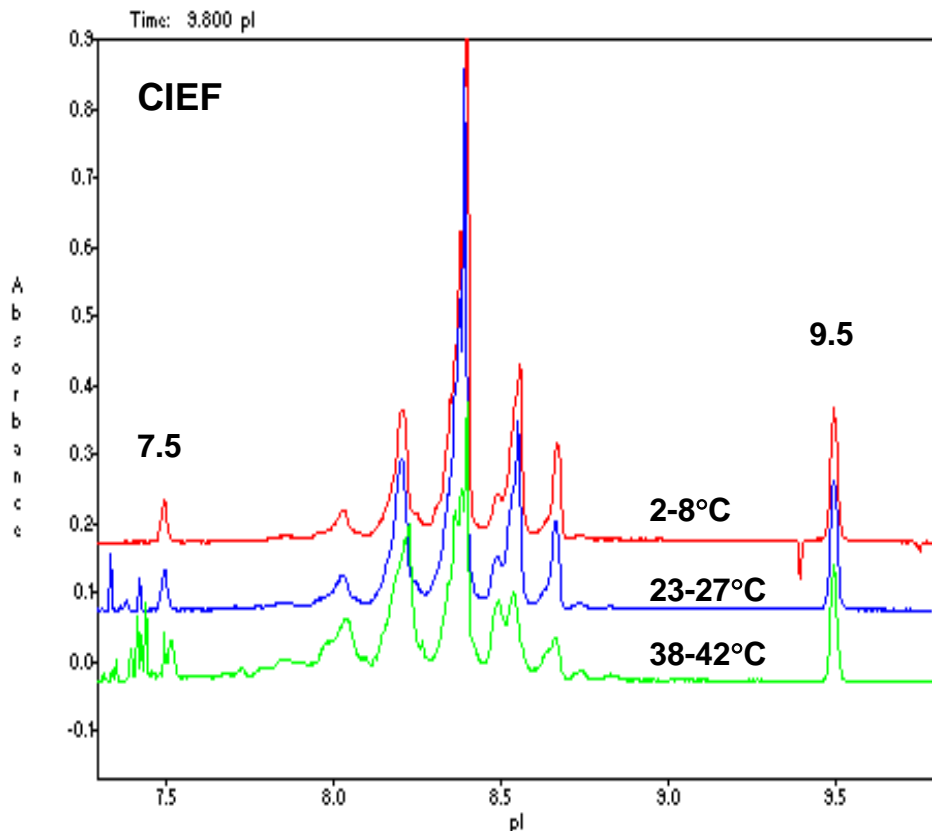
# Thermal Stability

## CASE 1: MAb3, 6 months

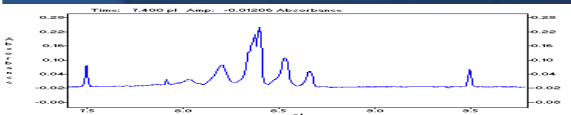

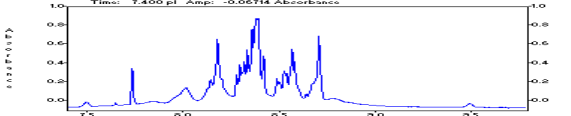

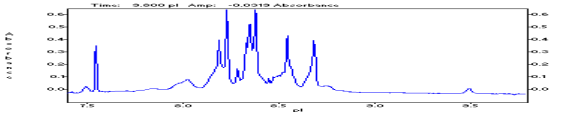

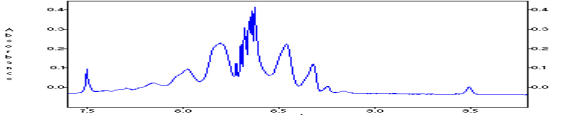

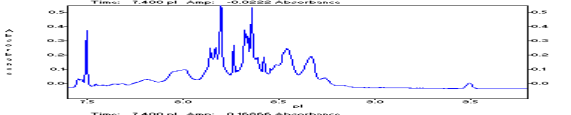

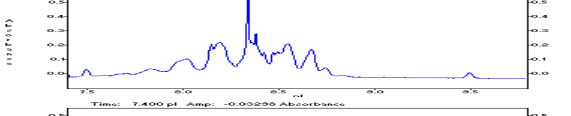

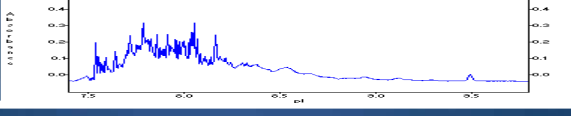



# Thermal Stability

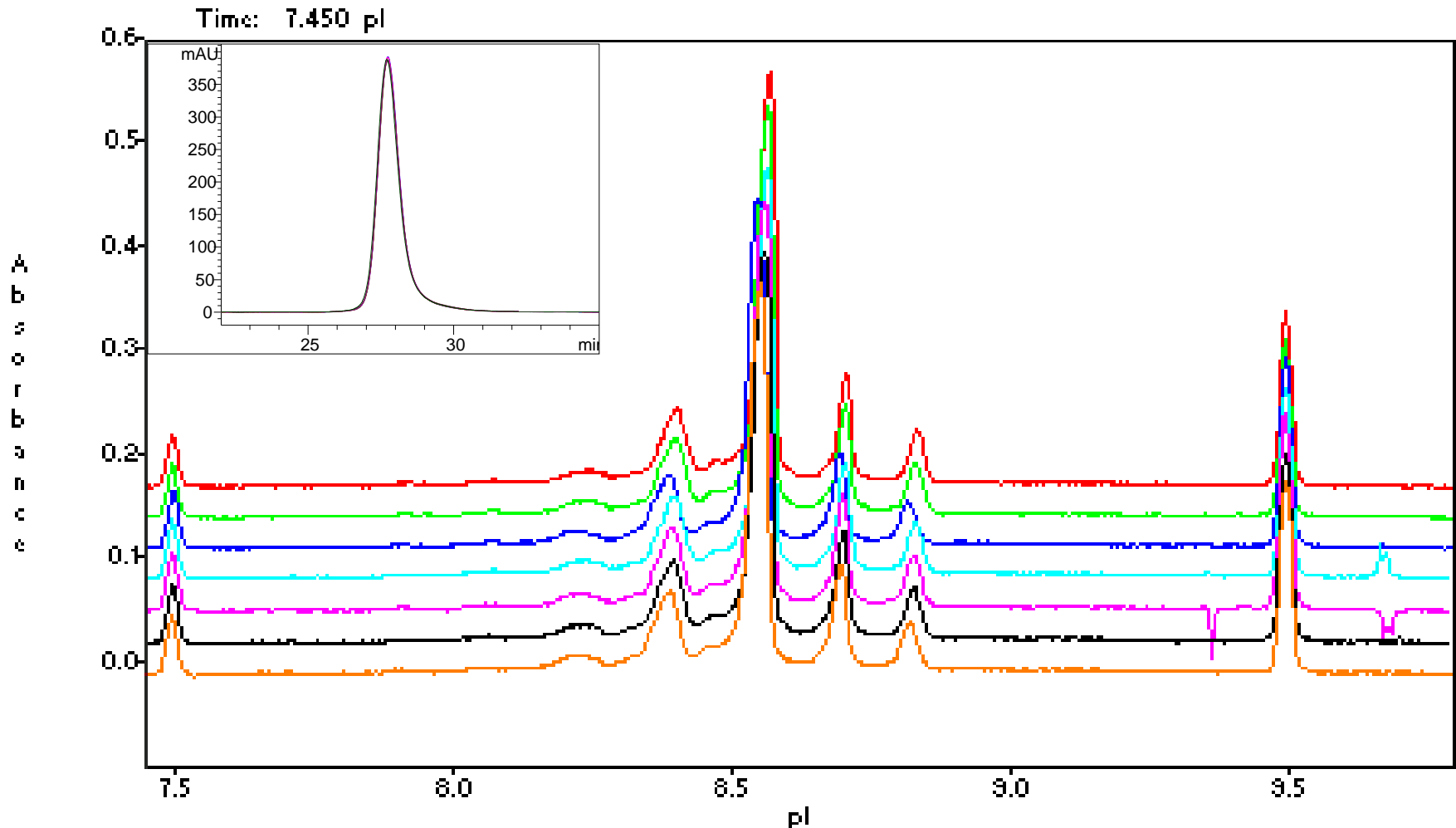
## CASE 2: MAb2, 21 months



# Correlation Between Analytical Assays and Biological Assay

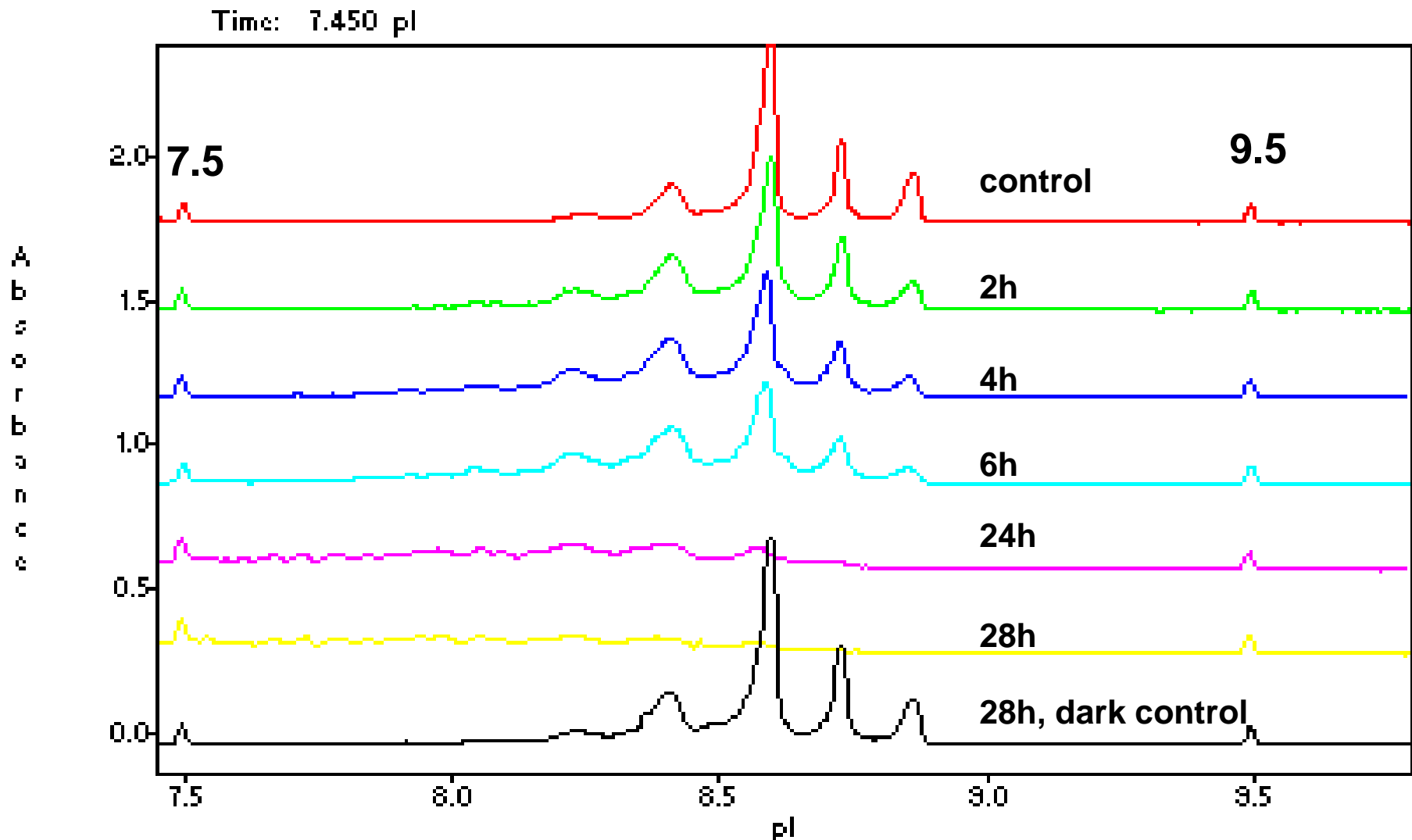
	Storage Temp (°C)	Time (Month)	CIEF Electropherogram	SDS-PAGE Reducing H L	ELISA Binding Assay (%)
1	2-8	NA			100
2	2-8	12			103.8
3	23-27	1			87.2
4	23-27	3			96.0
5	23-27	6			90.2
6	38-42	1			91.1
7	38-42	6			82.8

# Hydrogen Peroxide Oxidation



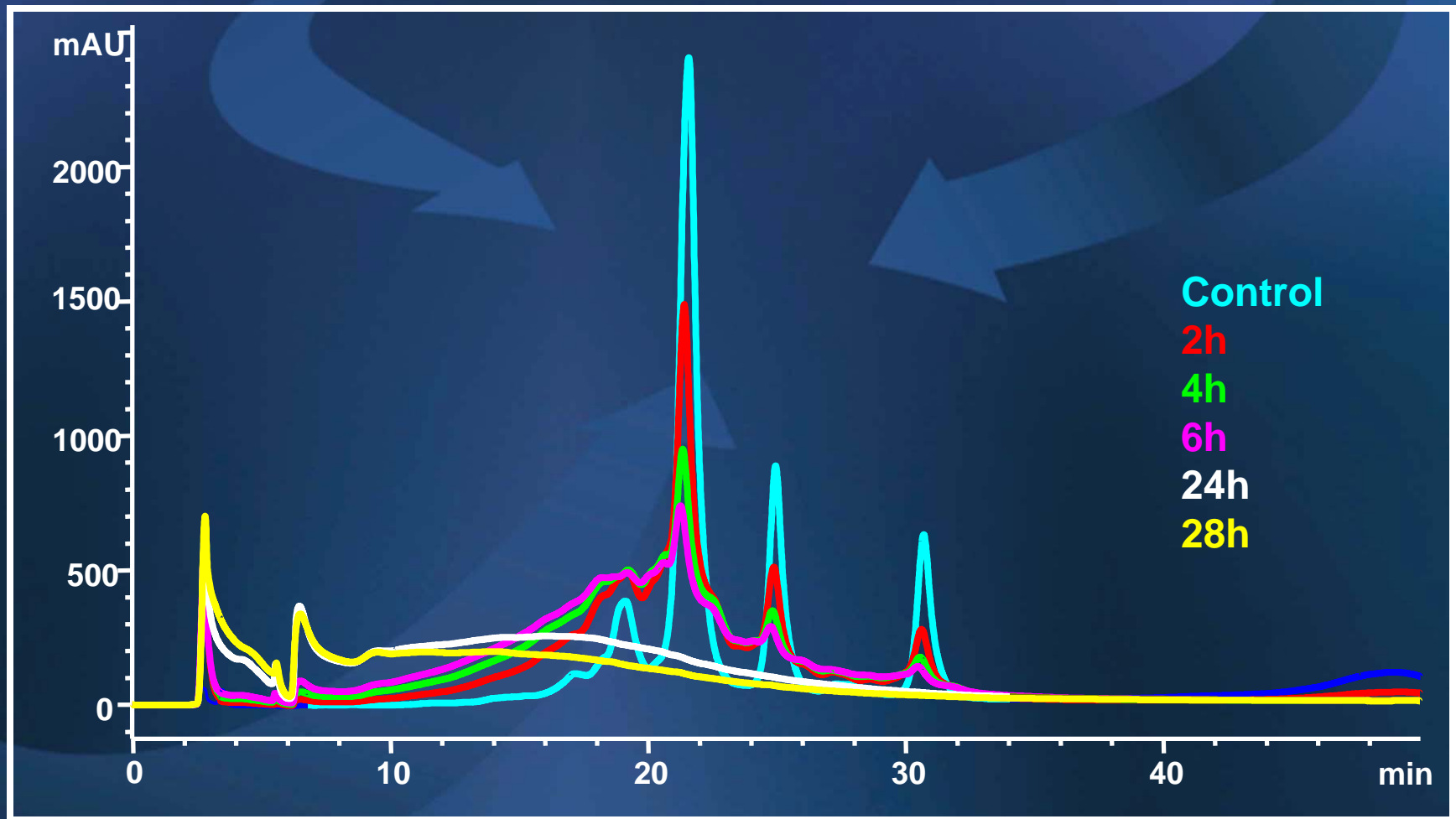
# Light-Induced Modification

## CASE : MAb1, CIEF



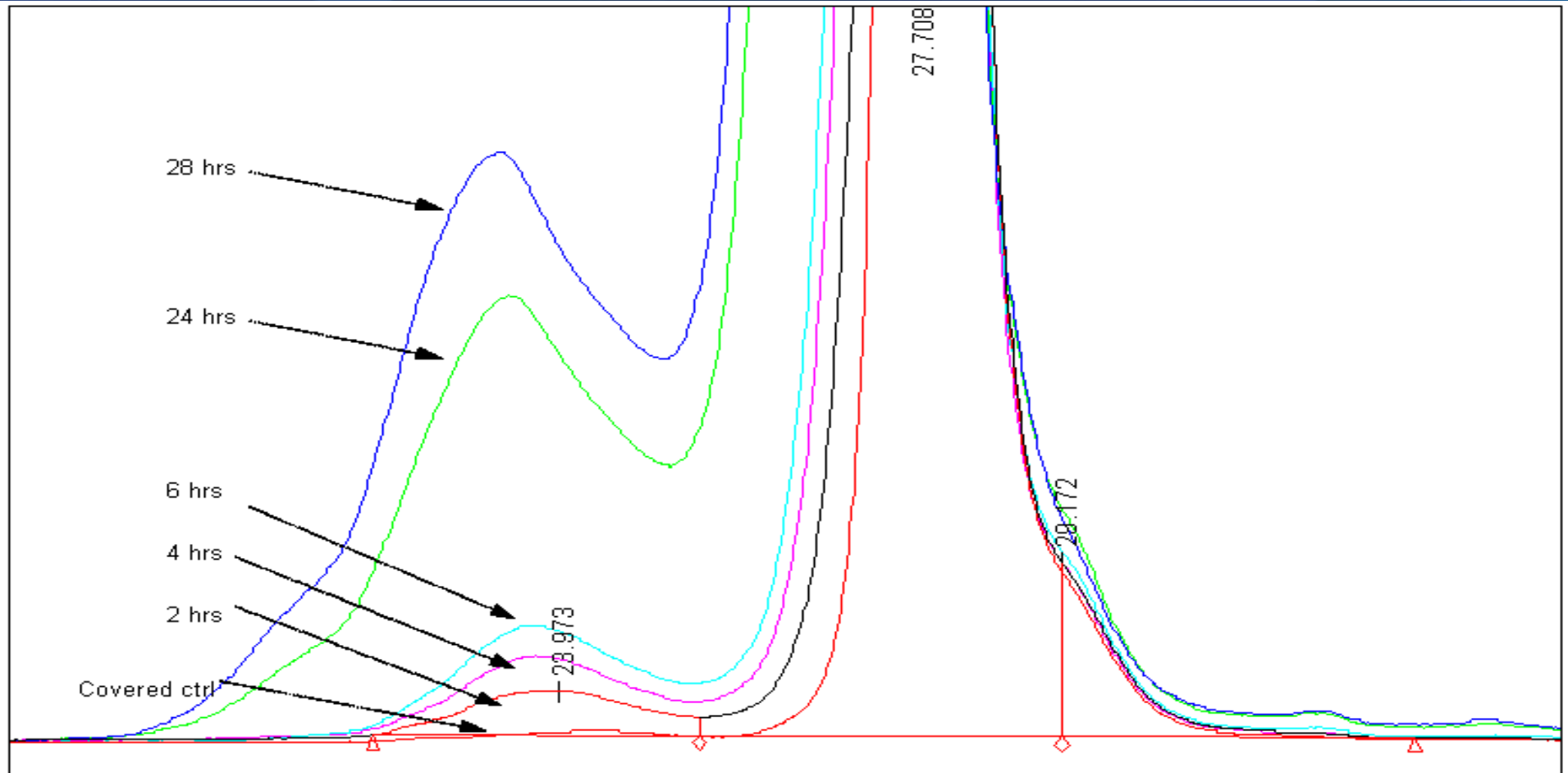
# Light-Induced Modification

## CASE 1: MAb1, IEC



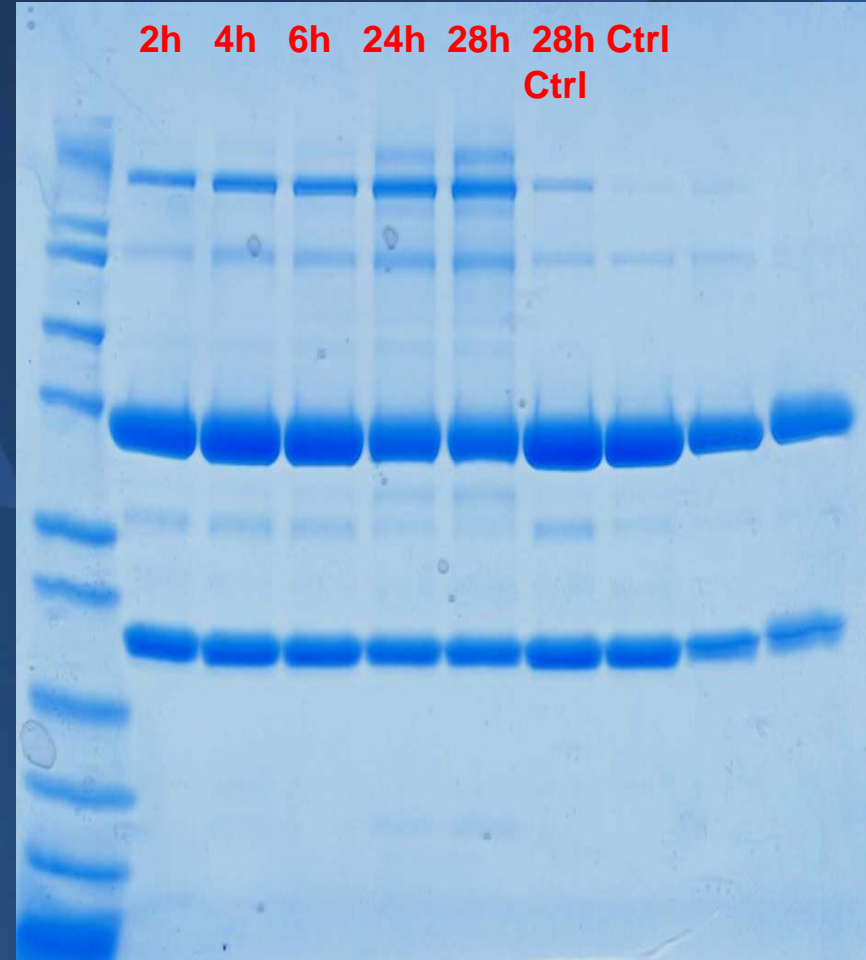
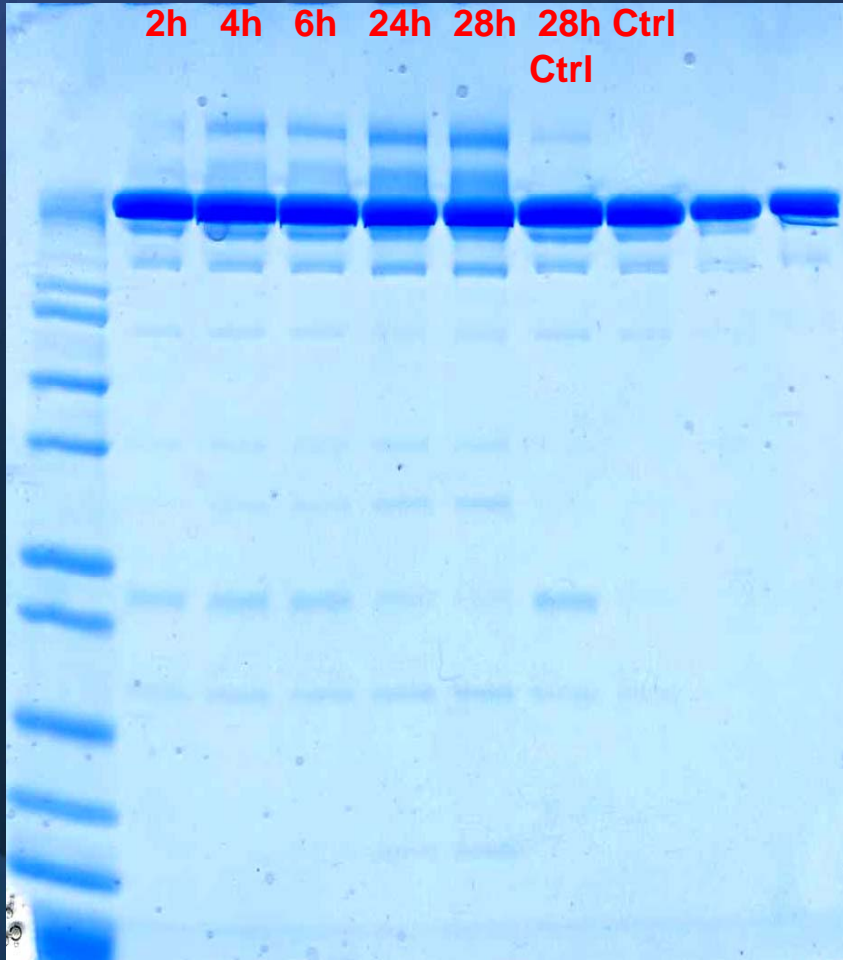
# Light-Induced Modification

## CASE 1: MAb1, SEC



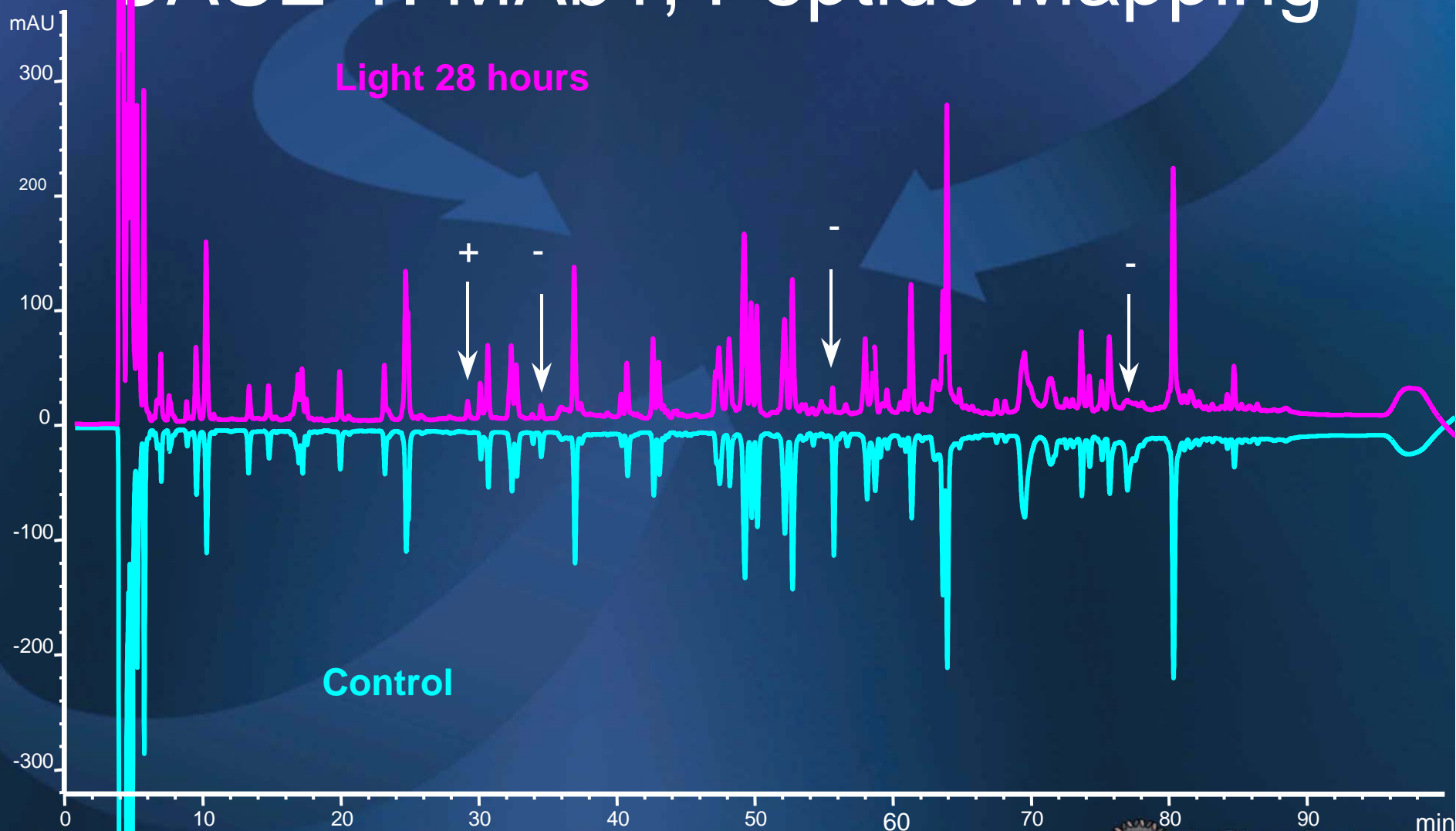
# Light-Induced Modification

## CASE 1: MAb1, SDS-PAGE



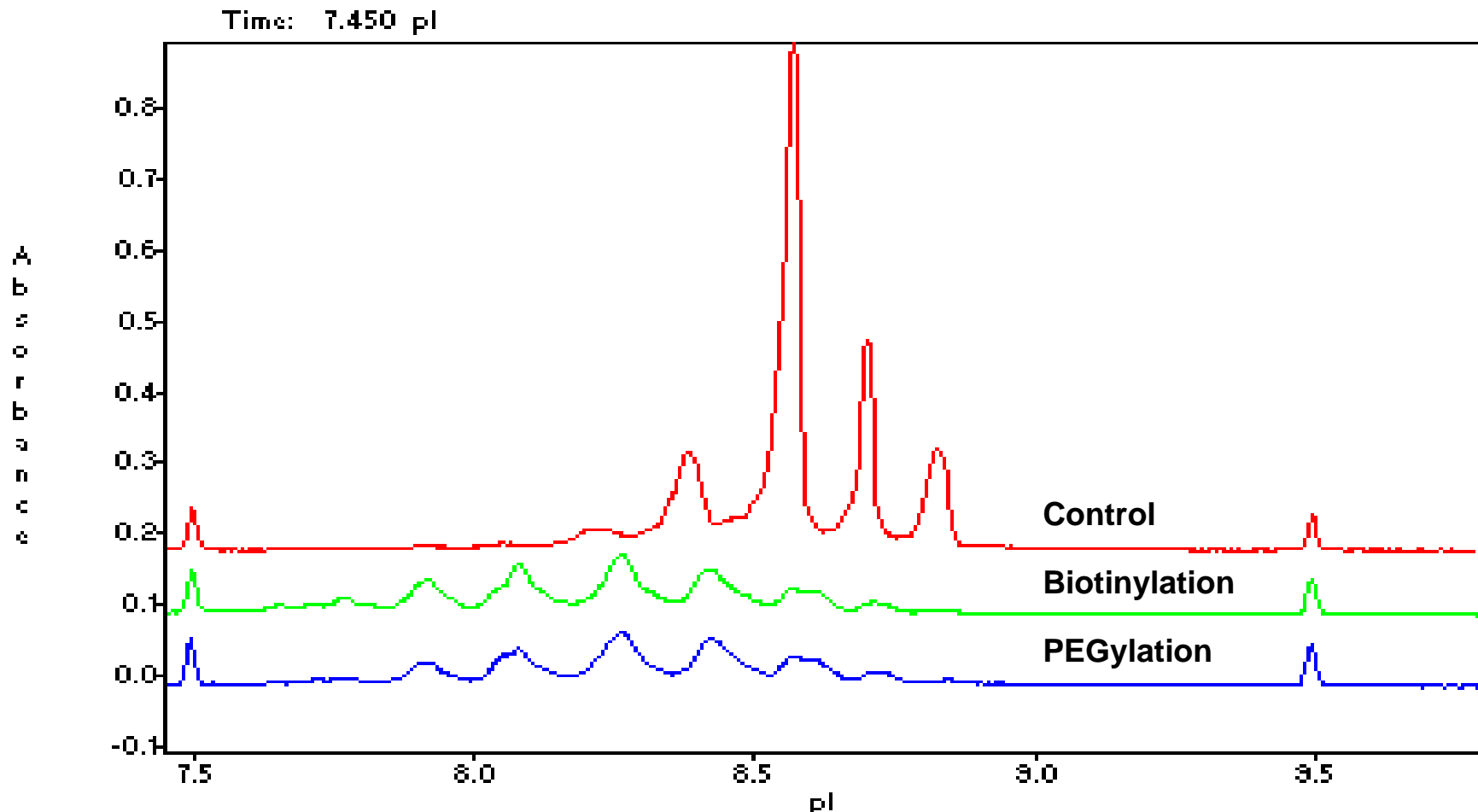
# Light-Induced Modification

## CASE 1: MAb1, Peptide Mapping



Antibody	Peptide Number	Sequence	Source	Theoretical Mass*	Observed Mass
MAb1	T20	DTLM <sup>+16</sup> ISR	HC	851.43	851.42
MAb1	T34	EEM <sup>+16</sup> TK	HC	653.28	653.27
MAb1	T33-T34	EPQVYTLPPSREEM <sup>+16</sup> TK	HC	1920.94	1920.93
MAb1	T33-T34	EPQVY <sup>+16</sup> TLPPSREEMTK	HC	1920.94	1920.93
MAb1	T22	FNWYVDGVEVH <sup>+16</sup> NAK	HC	1693.80	1693.79
MAb1	T22	FNW <sup>+4</sup> YVDGVEVHNAK	HC	1681.80	1681.79
MAb1	T22	FNW <sup>+4</sup> YVDGVEVH <sup>+16</sup> NAK	HC	1697.80	1697.79
MAb1	T5	GLEWVAVIW <sup>+16</sup> YDGSNK	HC	1752.86	1752.85
MAb1	T5	GLEWVAVIW <sup>+4</sup> YDGSNK	HC	1740.86	1740.85
MAb1	T25	VVSVLTVLHQDW <sup>+16</sup> LNGK	HC	1824.00	1823.99
MAb1	T25	VVSVLTVLHQDW <sup>+4</sup> LNGK	HC	1812.00	1811.99
MAb1	T25	VVSVLTVLH <sup>+16</sup> QDWLNGK	HC	1824.00	1823.99

# Chemical Manipulation of Monoclonal Antibodies



Techniques	Sample Preparation	Run Time	Wash Time	Data Process
WCID-CIEF	Desalt	~ 13 min	1.5 min	3 min/sample
Ion Exchange	Desalt	50 min	15 min	3 min/sample
Size Exclusion	NA	50 min	15 min	3 min/sample
SDS-PAGE	Heat 2-5 min	60-90 min	Staining Destaining ~2 h	Densitometer Scanning 10 min/sample
Peptide Mapping	Denature Reduction Alkylation Digestion, 24 hours	90-120 min	20 min	Chromatogram Mass spectra 30 min/sample

# Conclusions

- WCID-CIEF is a sensitive tool to catch most of the protein modifications, including sialylation, C-terminal lysine truncation, heat-induced modification, deamidation, light-induced oxidation and aggregation, and other manipulations such as biotinylation and pegylation.
- The assay itself is easy and quick compared to other analytical techniques.
- It is suitable for first-line bioanalytical, stability and formulation studies.

# Acknowledgements

## Bioanalytical Science

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