Benefits of Capillary Electrophoresis (CE) instrumentation in monoclonal antibody analysis

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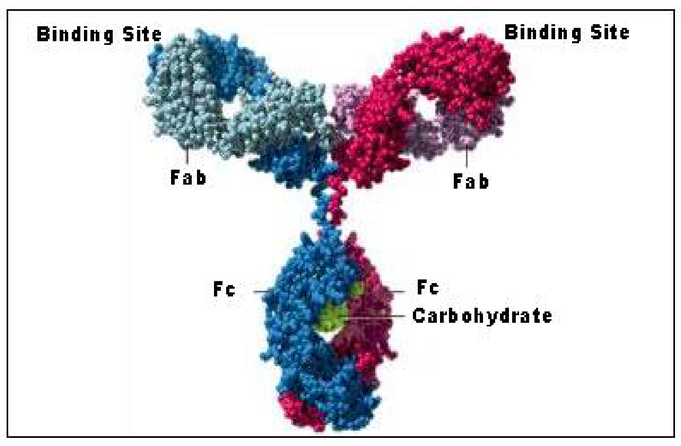
Introduction

- R&D work on monoclonal antibodies (mAb) within an NHS QC laboratory presents numerous challenges. Studies are high cost in terms of analysis and drugs.
- mAbs are large complex proteins and require a range of complex analytical techniques to assure chemical and physical stability.
- mAb purity has traditionally been assessed using Sodium Dodecyl Sulfate-Polyacrylamide Electrophoresis (SDS-PAGE), whilst charge heterogeneity analysis has used Ion Exchange Chromatography (IEC).
- Capillary Electrophoresis (CE) instrumentation can be used in different modes to characterise the purity and charge profile of proteins.
- CE-SDS has emerged as a replacement to SDS-PAGE due to its quantitative nature, on-line UV detection, automation and enhanced resolution of small proteins.
- Capillary Zone Electrophoresis (CZE) can be used to assess charge heterogeneity analysis of proteins.

Objective

The objective was:

- to provide a direct comparison of SDS-PAGE and CE-SDS analysis by assaying the same analytical mAb samples using both techniques.
- to evaluate the use of CZE in providing information on the charge heterogeneity of mAb biosimilars.



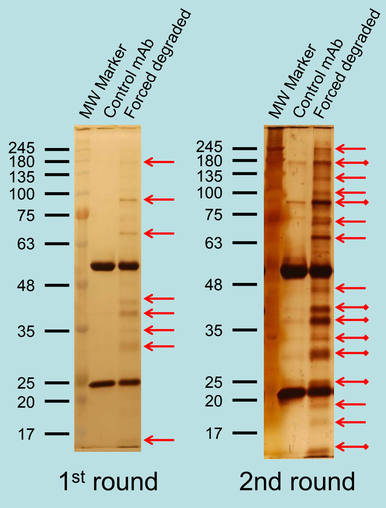
Methods

- An originator mAb (mAb-1) and its biosimilar (mAb-2) were reconstituted and diluted according to the relevant Summary of Product Characteristics. Aged mAb-1 preparations which had been stored long term at 2-8°C or 25°C/60%RH were also assessed.
- SDS-PAGE used the OmniPage Mini Vertical Electrophoresis System. Proteins ran under reducing conditions were detected by two rounds of silver staining of the gel using 0.2% w/v silver nitrate solution. Proteins ran under non-reducing conditions were detected by one round of Coomassie Brilliant Blue Colloidal Solution overnight followed by one round of silver staining. The suitability of the staining procedures was assessed using a control (2-8°C storage) and forced degraded (40°C storage) mAb.
- CE-SDS and CZE were performed using a deltaDOT High Performance Capillary Chromatography (HPCE-512TC) platform. CE-SDS was carried out under reducing and non-reducing conditions. All protein detection was at 214nm by diode array.

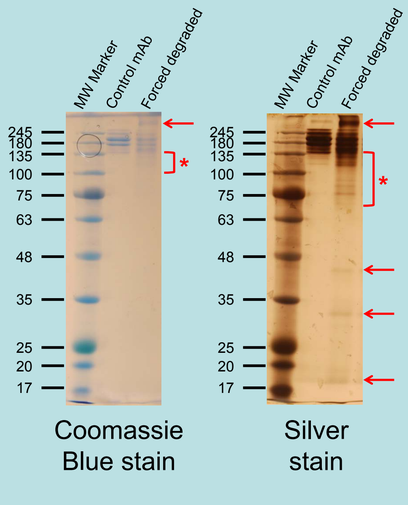
Results & Discussion

Improving sensitivity of SDS-PAGE protein detection

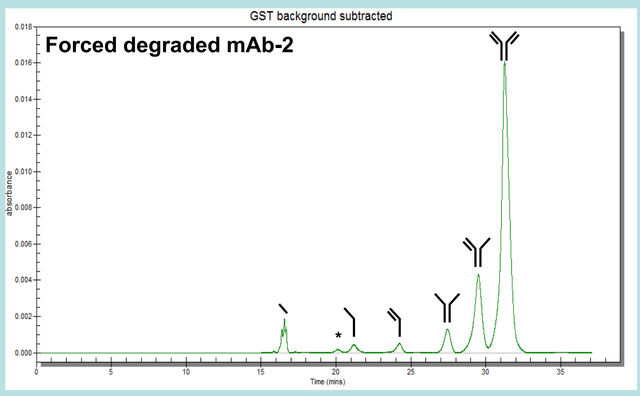
- **Reducing conditions:** One round of silver staining allowed confirmation of the molecular weight of the heavy and light chains (~50 and 25kDa, respectively) without saturated staining, and the presence of additional protein bands in the forced degraded samples (←).
- A second round of silver staining confirmed the presence of some of these additional bands in the control mAb sample, albeit at much lower levels (♦), and the presence of further additional bands in the forced degraded sample. Protein smearing was also evident in the forced degraded sample which is indicative of protein degradation.



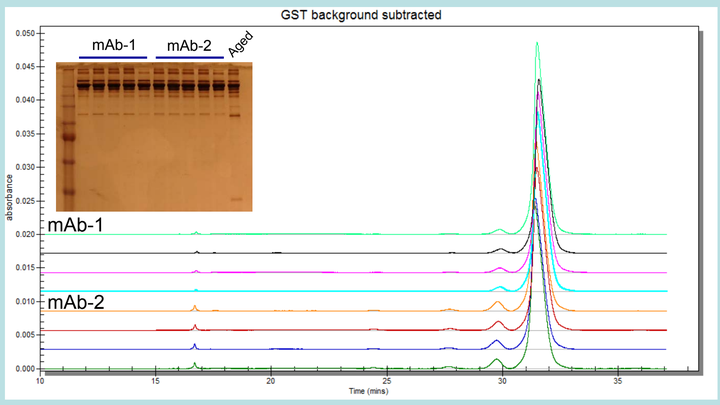
- **Non-reducing conditions:** Coomassie Blue staining allowed definition of the closely related size-variants, without saturation of the protein bands. An additional protein band (←) and evidence of protein smearing (*) were detected in the forced degraded sample.
- Subsequent staining with silver stain detected lower molecular weight proteins and more intense protein smearing in the forced degraded sample which is indicative of protein degradation.



Non-reducing CE-SDS vs SDS-PAGE



- **Non-reducing CE-SDS** analysis demonstrated the stability indicating ability of the method to separate the intact antibody from impurities such as the light chain, heavy chain, heavy-light chain, heavy-heavy chain and 2-heavy-1-light chain following thermal-induced fragmentation of the mAb. * indicates non-glycosylated heavy chain.
- **Silver staining** detected multiple size variants and subtle differences between mAb-1 and mAb-2 by SDS-PAGE.
- **Non-reducing CE-SDS** analysis of 4 different manufactured batches each of mAb-1 and mAb-2 showed good reproducibility for retention time and peak area.



➢ **CE-SDS allowed quantification of different fragments and detection of non-glycosylated species that cannot be distinguished by SDS-PAGE.**

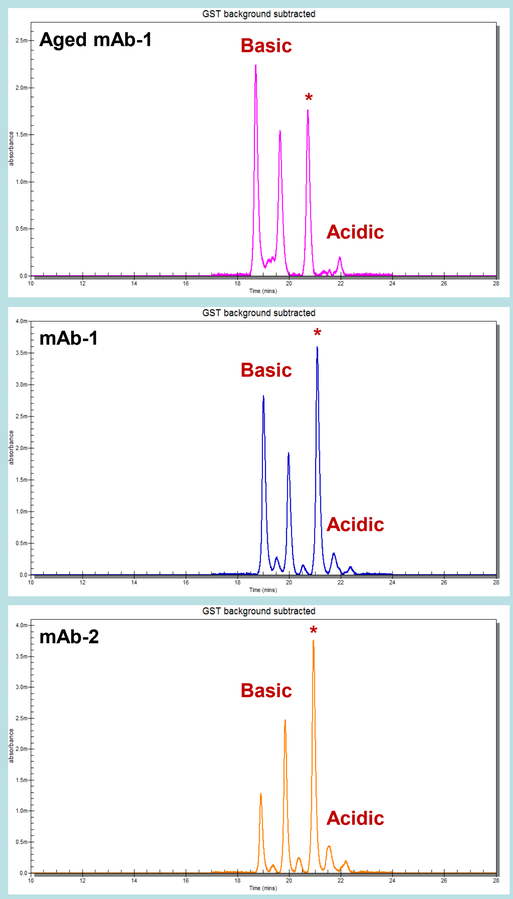
mAb	Non-reduced CE-SDS			
	Monomer peak			
	Retention time (min)	%RSD	% Area	%RSD
mAb-1	31.5	0.1	94.8	0.7
mAb-2	31.4	0.1	91.1	0.9

CZE

- **CZE** separates proteins based on their charge and hydrodynamic radius.
- CZE was used to monitor the charge heterogeneity between two brands of a mAb (mAb-1 and mAb-2) and Aged mAb-1 which had been stored long term at 2-8°C.
- Peaks were grouped as either Basic, Main or Acidic variants.

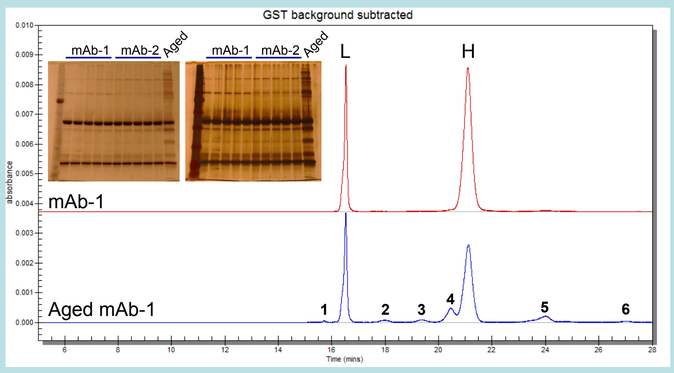
➢ **CZE detected the changes in the charge profile of mAb-1 due to degradation following long-term storage at 2-8°C.**

➢ **Distinct differences in the charge profile of the different mAb brands were detected.**



Peak ID	% Peak Area		
	Basic	Main peak (*)	Acidic
Aged mAb-1	70.3	25.4	4.3
mAb-1	56.7	36.7	6.6
mAb-2	47.3	41.6	11.1

Reducing CE-SDS vs SDS-PAGE



- **Double silver staining** increased the sensitivity for detecting differences between the mAb-1 and mAb-2 by SDS-PAGE. Multiple protein bands and smearing was evident in the Aged mAb-1 which is indicative of degradation.
- **Reducing CE-SDS** analysis allowed quantification of the heavy and light chains and degradents in the Aged mAb sample. Non-glycosylated heavy chain (Peak 4) was also detected.

mAb	Reduced CE-SDS							
	Peak							
	Heavy chain (H)	Light chain (L)	1	2	3	4	5	6
mAb-1	65.0	31.0	0.1	0.5	0.7	1.1	1.5	0.1
Aged mAb-1	47.0	33.5	0.5	1.8	2.0	8.4	6.1	0.8

Conclusions

➢ **Double staining techniques** can greatly improve the sensitivity of **SDS-PAGE** and is appropriate as a qualitative approach to detect gross changes in mAbs.

➢ **CE-SDS** enables quantification of fragments and non-glycosylated species, providing an invaluable tool for monitoring stability and release testing.

➢ **CZE** detected differences in charge variants in different mAb brands and through degradation and could have uses in identifying counterfeit mAbs.

➢ **CE instrumentation** can be applied in different modes to allow purity and charge analysis with high resolution and repeatability and is a viable replacement for SDS-PAGE and IEC in QC labs for stability analysis and release testing.

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