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# Elucidation of Mg2+ Induced Size and Charge Heterogeneity in Monoclonal Antibody Therapeutics

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# Introduction

- Monoclonal antibodies (mAbs) are essential therapeutics, but their stability is affected by aggregation and charge heterogeneity.
- Charge variants arise from chemical modifications like deamidation, oxidation, and isomerization, impacting bioactivity and shelf life.
- Metal ions such as magnesium ($Mg^{2+}$) are commonly introduced during production, storage, and formulation processes.
- Although $Mg^{2+}$ is biologically abundant, its effects on mAb structural stability are not fully understood.
- Some studies suggest $Mg^{2+}$ may destabilize mAbs by promoting aggregation and chemical modifications.
- This study evaluates the impact of $Mg^{2+}$ contamination (0.5 mM $MgSO_4$ at 37 °C) on the size and charge heterogeneity of a trastuzumab biosimilar.
- Trastuzumab samples are incubated in 3 different conditions (25 °C, 37 °C and 37 °C with 0.5 mM MgSO4 for a period of 4 weeks to assess the modifications in duplicates.
- Orthogonal analytical techniques (SEC-UV, CEX-MS, native MS, peptide mapping) were used to characterize stress-induced changes.

# Experimental

## Samples:

- Trastuzumab (in-house IgG1 biosimilar)
- Rituximab (used for comparative analysis)

## Stress Conditions:

- Control: 25 °C
- Thermal stress: 37 °C (4 weeks)
- $Mg^{2+}$ stress: 0.5 mM $MgSO_4$ at 37 °C (4 weeks)

## Analytical Techniques:

- Size-Exclusion Chromatography (SEC-UV/MS): Native MS analysis was conducted to quantify aggregates and fragments. This involved the use of an Agilent 1290 Infinity II system, coupled with a 6545XT AdvanceBio LC/Q-TOF.
- Cation-Exchange Chromatography (CEX-UV/MS): To detect acidic and basic variants, native MS analysis was performed using an Agilent 1290 Infinity II system, integrated with a 6545XT AdvanceBio LC/Q-TOF.
- Reverse-Phase Liquid Chromatography (RP-LC-MS/MS): Peptide mapping analysis was carried out following trypsin digestion. The workflow included RP-LC separation and MS/MS analysis, executed with the Agilent 1290 Infinity II system, coupled to a 6545XT AdvanceBio LC/Q-TOF, to identify site-specific modifications. Details can be found in reference 1

# Results and Discussion

| Sample | Acidic % | | | | | Main % | Basic % | |
|---|---|---|---|---|---|---|---|---|
| | A5 | A4 | A3 | A2 | A1 | M | B1 | B2 |
| Control | 32.23 % | | | | | 52.63 % | 15.14 % | |
| | – | 148,065.2 | 148,060.5 | 148,062.5 | 148,061.8 | 148,058.3 | 148,058.3 | 148,060.3 |
| Thermal | 60.87 % | | | | | 24.55 % | 14.52 % | |
| | 148,066.0 | 148,064.0 | 148,060.6 | 148,060.2 | 148,057.0 | 148,055.7 | 148,055.4 | 148,063.7 |
| Metal | 62.61 % | | | | | 24.0 % | 13.30 % | |
| | 148,067.9 | 148,065.7 | 148,063.3 | 148,061.1 | 148,059.8 | 148,058.5 | 148,057.7 | 148,060.4 |

Summary of deconvoluted mass result (Da) of charge variants of control and stressed mAb sample

# Results and Discussion

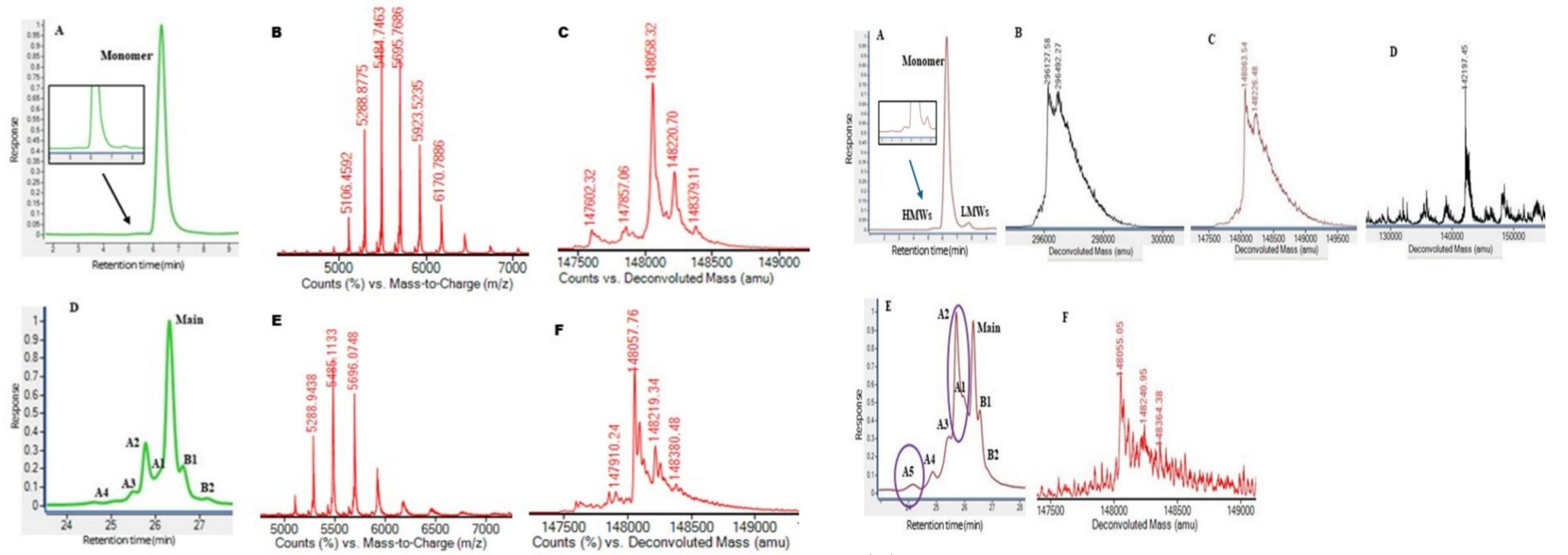


Fig. 1: Characterization of trastuzumab biosimilar (control). (A) SEC chromatogram of trastuzumab (inset figure: baseline zoom for SEC profile showing monomer); (B) Native MS spectra of main peak of trastuzumab; (C) Deconvolution mass result of main peak of trastuzumab; (D) CEX chromatogram of trastuzumab; (E) Native MS spectra of main peak of trastuzumab in the CEX-MS analysis; (F) Deconvoluted mass of main peak.

(A) SEC chromatogram of thermal stressed trastuzumab sample (inset figure: baseline zoomed UV profile for better visualization of aggregates and fragments); (B) Deconvolution mass result of high molecular weight (HMWs) peak; (C) Deconvolution mass result of main peak of stressed sample; (D) Deconvolution mass result of low molecular weight (LMWs) peak; (E) CEX chromatogram of thermal stressed trastuzumab sample. The changes in acidic variant vis-à-vis control are highlighted by purple circle along with the presence of a new peak (variant A5); (F) Deconvolution result of main peak of stressed sample.

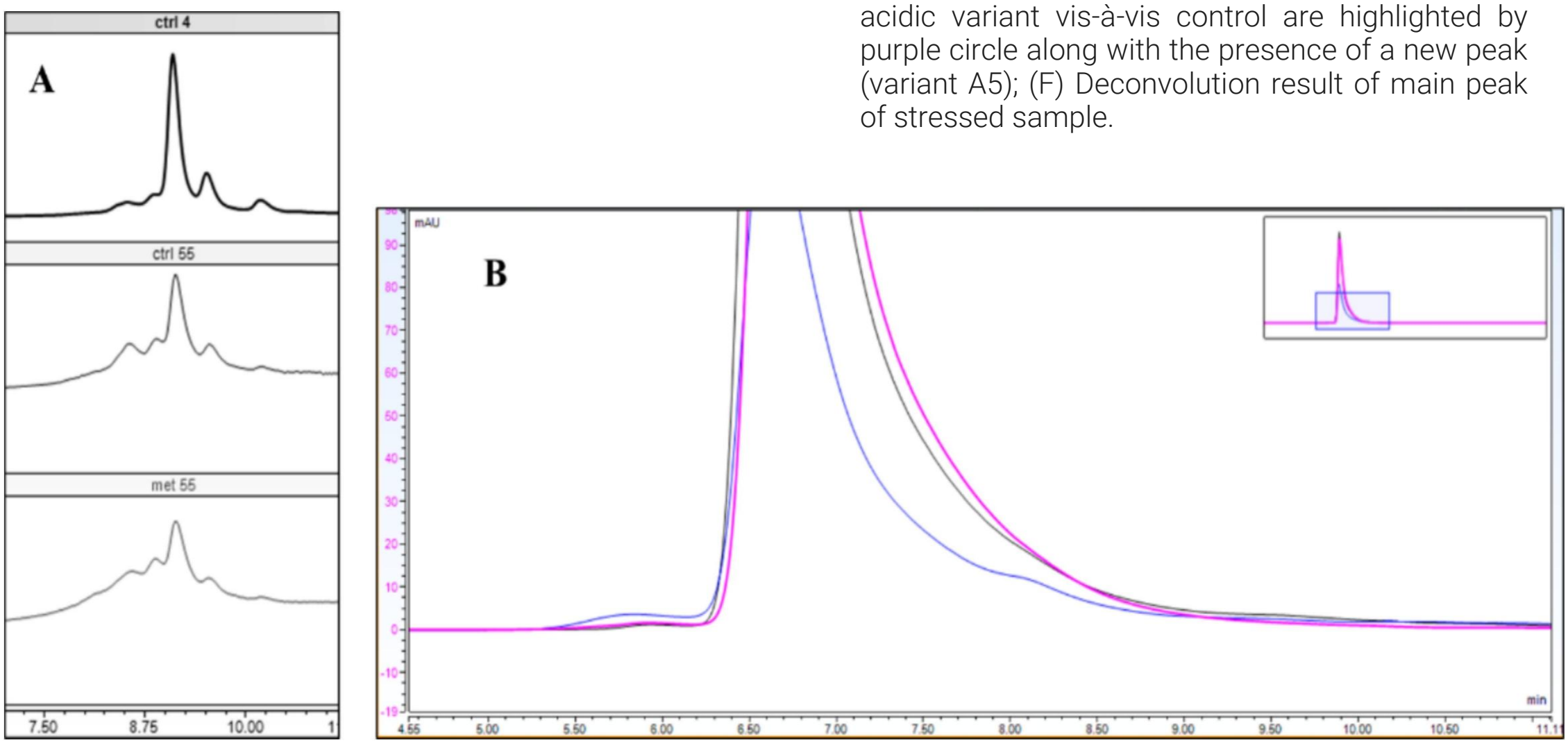


Fig. 2: A) CEX-UV (top: control; middle: thermal; bottom: metal stressed sample) and B) SEC-UV profile of $Mg^{2+}$ stressed rituximab sample (black: control; pink: thermal; blue metal sample). We see an increment in acidic species and aggregates for the metal samples compared to control.

# Results and Discussion

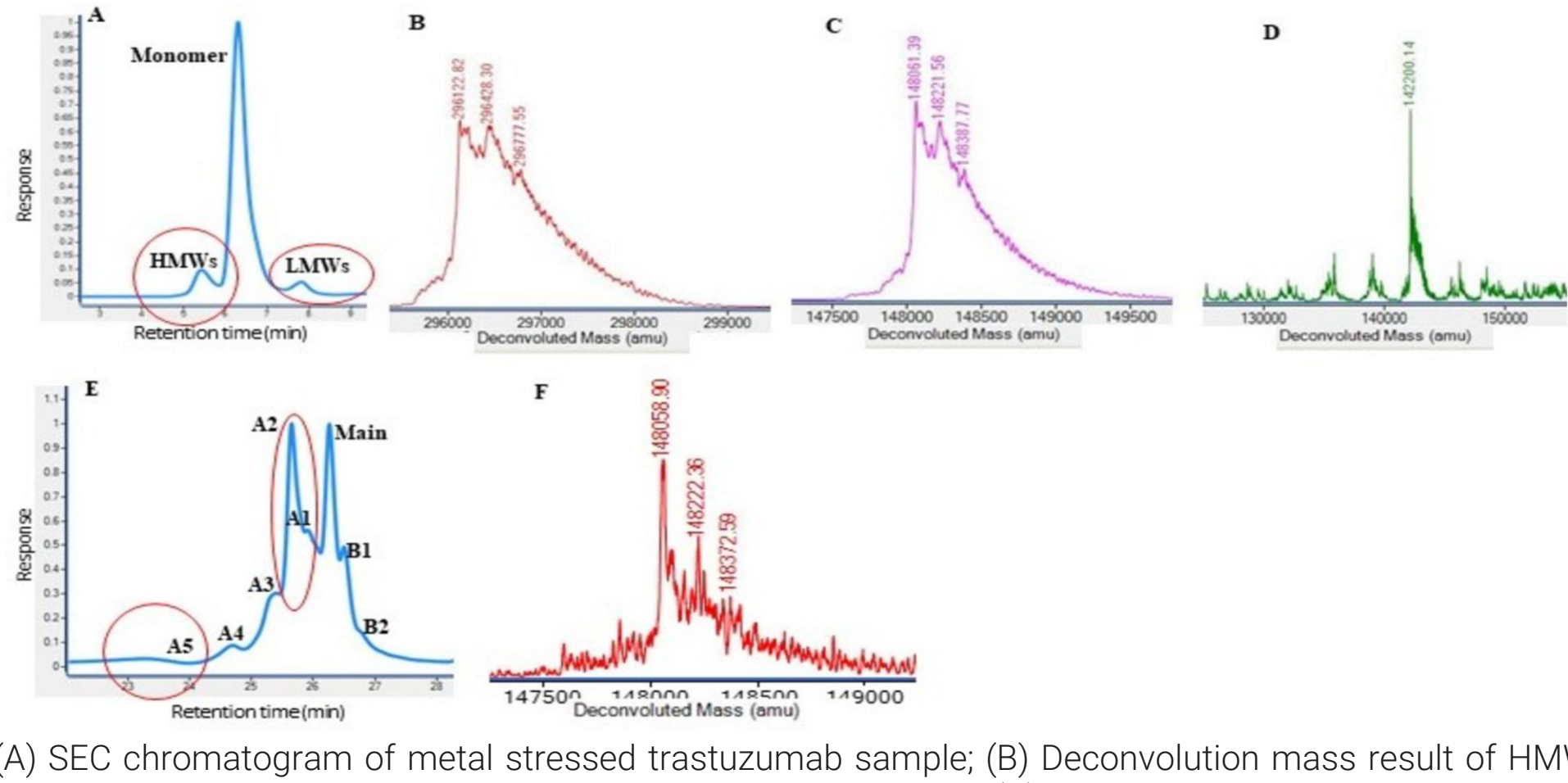


Fig. 3: (A) SEC chromatogram of metal stressed trastuzumab sample; (B) Deconvolution mass result of HMWs peak; (C) Deconvolution mass result of main peak of metal stressed sample; (D) Deconvolution mass result of LMWs peak; (E) CEX chromatogram of metal stressed trastuzumab sample.; (F) Deconvolution result of main peak of stressed sample. The increment in HMWs, LMWs and acidic variant with respect to control sample are highlighted in red circle along with the additional acidic peak (variant A5)

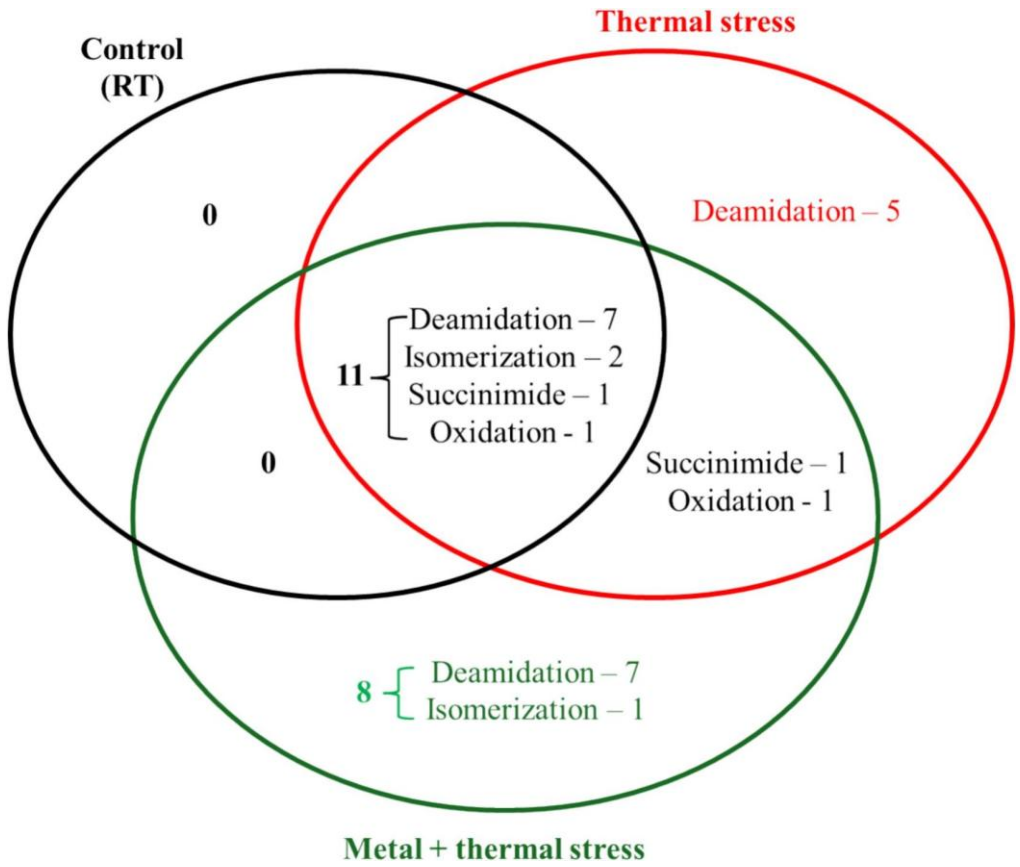


Fig. 4: Summary of post-translational/chemical modification distribution of trastuzumab. The black circle represents modification identified in control sample, the red circle represents modifications identified in thermal stressed sample, and the green circle represents modifications identified in metal stressed sample of trastuzumab.

## Conclusions

- $Mg^{2+}$ contamination increases aggregation (~9%) and acidic charge variants (~2 fold) in trastuzumab.
- Metal-induced stress leads to multiple chemical modifications including deamidation, oxidation, and succinimide formation.
- Key hotspots for modification include LC-N30 and HC-N55 (deamidation), HC-M256 (oxidation), and disrupted disulfide linkages.
- Similar degradation trends observed in rituximab confirm that $Mg^{2+}$ effects are not molecule-specific.
- Highlights the need for strict monitoring of metal ion levels during manufacturing and storage of mAbs.

## References


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