THE SGS M-SCAN CENTRE OF EXCELLENCE IN BIOPHYSICAL ANALYSIS

AN ORTHOGONAL APPROACH TO FORMULATION AND STABILITY STUDIES

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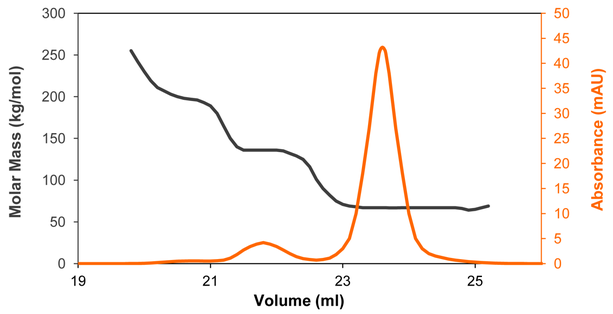
INTRODUCTION

- The use of Biophysical techniques in research and development is increasing rapidly
- **ICH Guideline Q6B:** 'The higher-order structure of the product is examined using procedures such as circular dichroism, nuclear magnetic resonance (NMR), or other suitable techniques, as appropriate'
- SGS M-Scan offers established GxP compliant absorbance, CD, DSC, AUC and SEC-MALS
- The company has recently extended its range of biophysical techniques, and now offers fluorescence, FTIR and DLS services
- We demonstrate here the application of SGS M-Scan's orthogonal analysis capabilities to the characterisation of biological material

SIZE ESTIMATIONS

FIGURE 1: SEC-MALS OF NATIVE BSA



- SEC-MALS (Fig. 1) uses absorbance, refractive index and multi-angle light scattering detection systems
- Allows accurate estimation of both the molar mass and relative concentration of each of the SEC-separated entities

TABLE 1: SUMMARY OF SEC-MALS DATA (FIG. 1)

	MONOMER	DIMER	TRIMER
Molar Mass (kg/mol)	65.5	134.6	207.9
Mass Fraction (%)	86.9	11.3	1.8

- AUC (Fig. 2) used to further characterise lower molecular weight oligomers, whilst DLS (Fig. 3) allows detection of trace amounts of aggregated material (Tab. 3)

FIGURE 2: AUC OF NATIVE BSA

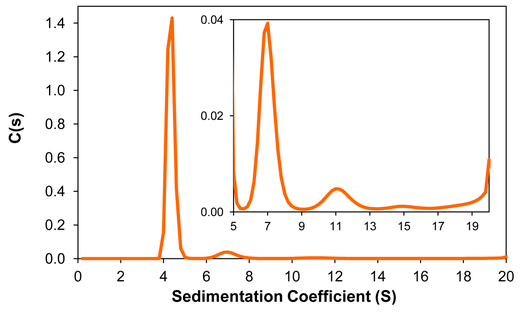


TABLE 2: SUMMARY OF AUC DATA (FIG. 2)

	MONOMER	DIMER	TETRAMER	HEXAMER
Sed. Co. (S)	4.4	7.0	11.0	15.0
MW (kDa)	58	119	239	371
% of total	92.4	5.8	1.4	0.4

FIGURE 3: DLS OF NATIVE AND THERMALLY STRESSED BSA – SIZE DISTRIBUTION BY VOLUME

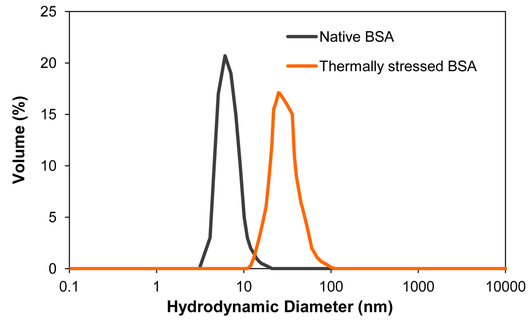


TABLE 3: DLS OF NATIVE BSA – SIZE DISTRIBUTION BY INTENSITY ANALYSIS

	PEAK 1	PEAK 2
Hydrodyn. Diam. (nm)	8.314	185.8
Intensity (%)	72.3	24.8

HIGHER ORDER STRUCTURE

- A comparability study on IgG in two different formulations is described below

FIGURE 4: FAR-UV CD OF IGG IN PBS AND TRIS FORMULATIONS

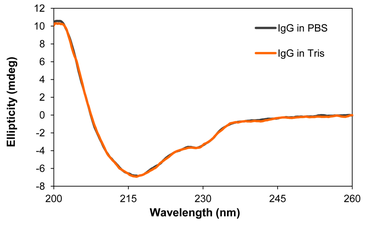
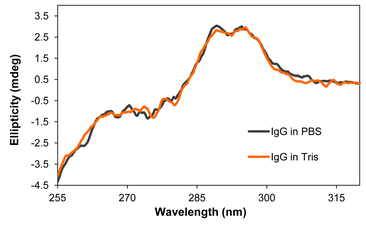


FIGURE 5: NEAR-UV CD OF IGG IN PBS AND TRIS FORMULATIONS



- CD detected no differences in the asymmetry of peptide bonds (Fig. 4) or aromatic residues (Fig. 5) between the materials

FIGURE 6: SECOND DERIVATIVE ABSORBANCE OF IGG IN PBS AND TRIS FORMULATIONS

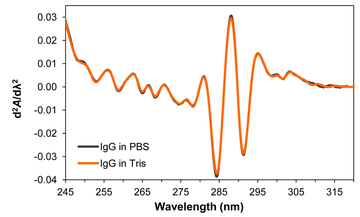
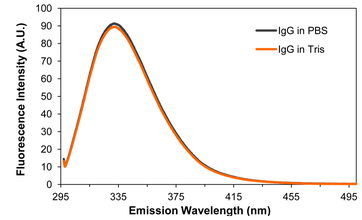


FIGURE 7: INTRINSIC FLUORESCENCE OF IGG IN PBS AND TRIS FORMULATIONS



- Absorbance (Fig. 6) and fluorescence spectroscopies (Fig. 7) detected no differences in aromatic residue environment
- Nevertheless, FTIR (Fig. 8) demonstrated contrast between the amide-associated hydrogen-bonding pattern of IgG in the two formulations, the β-sheet content in Tris being higher than in PBS

FIGURE 8: FTIR OF IGG IN PBS AND TRIS FORMULATIONS

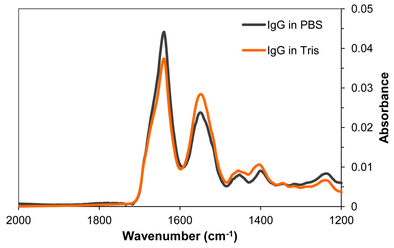
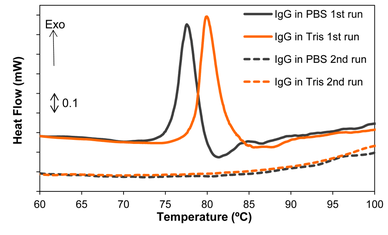


TABLE 4: FACTOR ANALYSIS OF FTIR SPECTRA (FIG. 8)

IgG Formulation	HELIX	SHEET
PBS	15 ± 4	34 ± 2
Tris	5 ± 3	40 ± 2

- DSC results (Fig. 9) also show a contrast, the melting temperature of IgG being 78.6 °C in PBS and 81.3 °C in Tris

FIGURE 9: DSC OF IGG IN PBS AND TRIS FORMULATIONS



CONCLUSIONS

- IgG in tris contains more sheet and less helix, and is also more stable, than IgG in PBS – consistent with the previous finding that IgG denaturation involves loss of sheet and gain of helix (Vermeer and Norde 2000)
- The rationale behind recent FDA recommendations on the use of an orthogonal biophysical approach to characterise biological materials is substantiated

REFERENCE

Vermeer, A. W. P. And Norde, W. (2000). Biophysical Journal 78: 394–404