Beta Cyclodextrin Derivatives as Protein Aggregation Modulators

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INTRODUCTION

Protein aggregation is the major challenge encountered during manufacturing, storage and transportation of biopharmaceuticals (1,2). Our objective was to evaluate the effect of two ßcyclodextrins derivatives: (KLEPTOSE® HPB hydroxypropyl-ß-cyclodextrin, with MS=0.65) and (KLEPTOSE® HP hydroxypropyl-ß-cyclodextrin, with MS=0.9) on two biologic drugs (Infliximab and Etanercept) aggregation using high-throughput formulation screening (*i*Formulate™) and nanoDSF (Differential Scanning Fluorimetry) (3,4).

MATERIALS & METHODS

Infliximab (Remicade®), a tumor necrosis factor (TNF-alpha) binding antibody (chimeric IgG1) and Etanercept (Enbrel®), a dimeric fusion protein were purchased (in PBS: phosphate saline buffer) from BOC Sciences. Simultaneous evaluation of Tm (melting temperature) and relative degree of aggregation of proteins in various molarity of KLEPTOSE® HPB and HP (0 mM, 0.125 mM, 0.25 mM, 1.25 mM, 2.5 mM, 5 mM, 10 mM, 25 mM, 50 mM, 100 mM and 200 mM) was performed using NanoDSF (Prometheus NT.Plex,Fig 1).

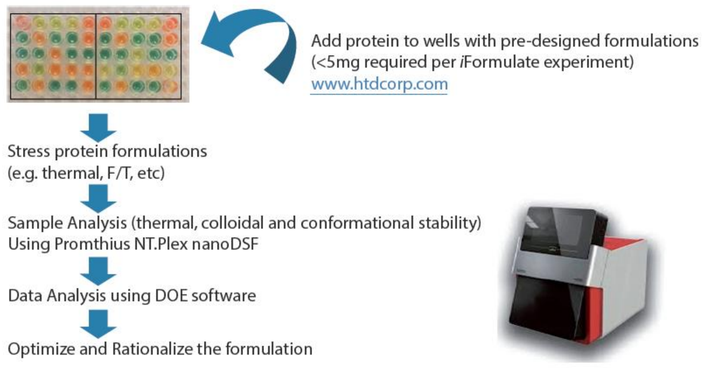


Fig. 1 : *i*Formulate™ Plate for High Throughput Formulation using DOE

RESULTS & DISCUSSION

Thermal and colloidal stability of 4 mg/mL Infliximab and 6mg/mL Etanercept in *i*Formulate® RS-2 plate are affected (Fig. 2A and Fig. 2B) by different formulation variables (pH, ionic strength, stabilizer conc., buffer conc.). Infliximab is much more prone to aggregation than Etanercept.

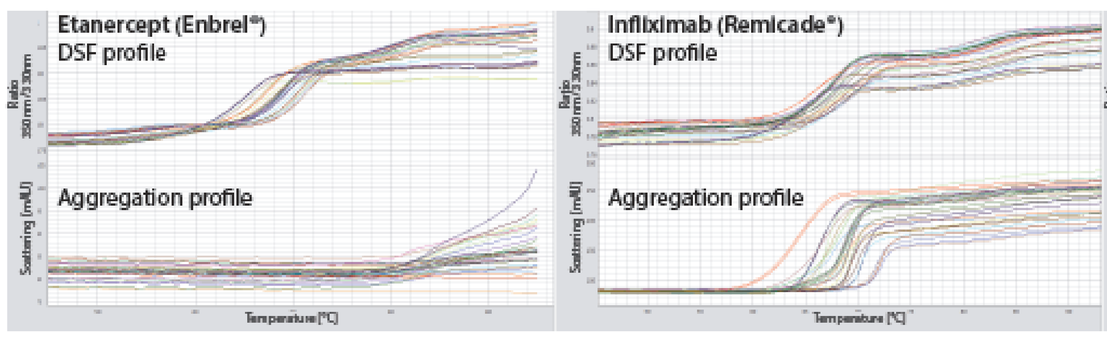


Fig. 2A : DSF and Aggregation profile of Infliximab and Etanercept in *i*Formulate™ (25 formulations tested simultaneously for thermal and colloidal stability).

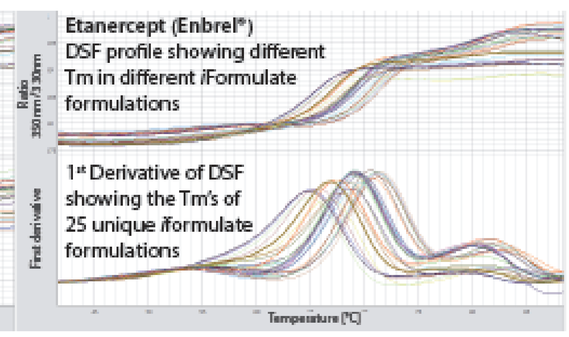


Fig. 2B : *i*Formulate™ on thermal stability of Etanercept (Enbrel®)

Pareto and DoE analysis of Etanercept in *i*Formulate® RS-2 plate formulations illustrate that pH dictates its thermal stability.

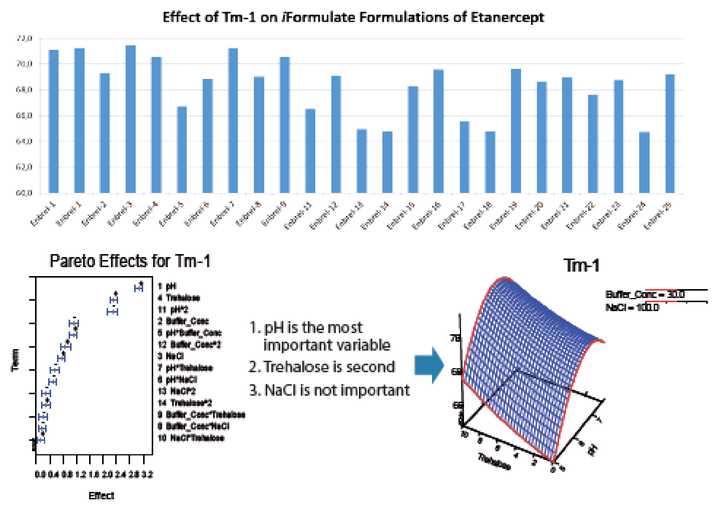


Fig. 3 : Etanercept (Enbrel®) Thermal stability by Pareto and DOE analysis of iFORMULATE™ FORMULATIONS

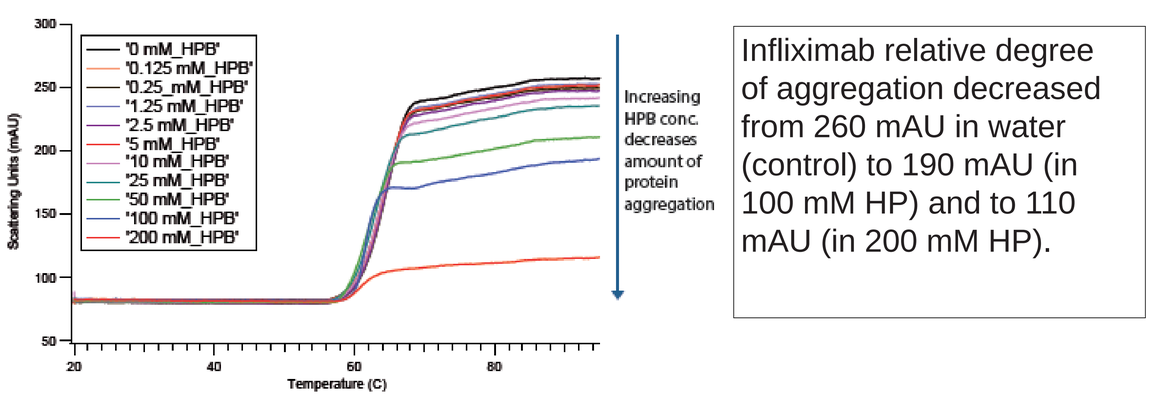
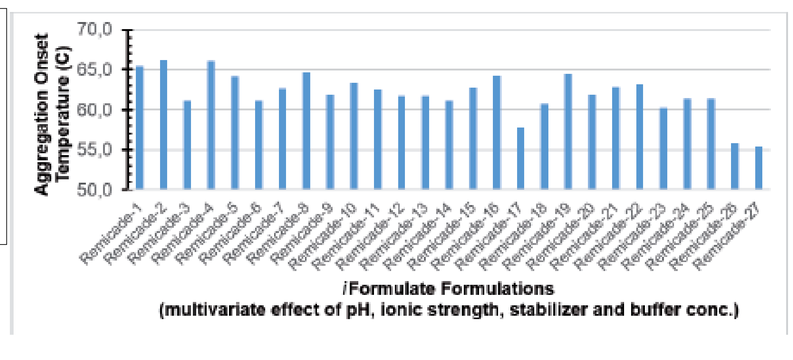


Fig. 5 : Infliximab (Remicade®) aggregation Decrease with increasing hpB molarities

Infliximab relative degree of aggregation decreased from 260 mAU in water (control) to 190 mAU (in 100 mM HP) and to 110 mAU (in 200 mM HP).

Infliximab's thermal stability by Pareto and DOE analysis of *i*Formulate™ formulations are ranking Trehalose ≥ NaCl > pH as critical variables.



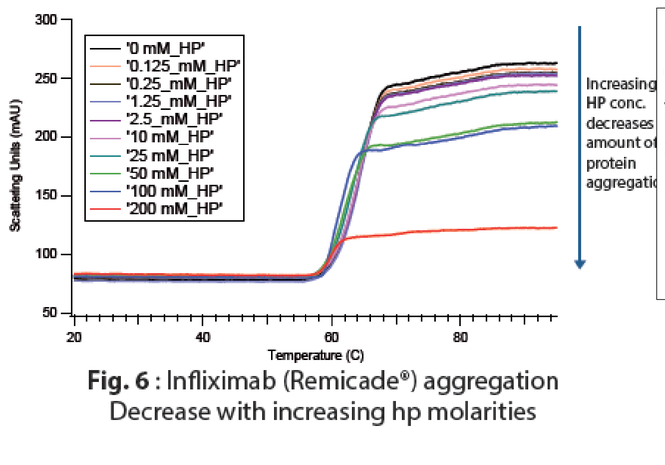
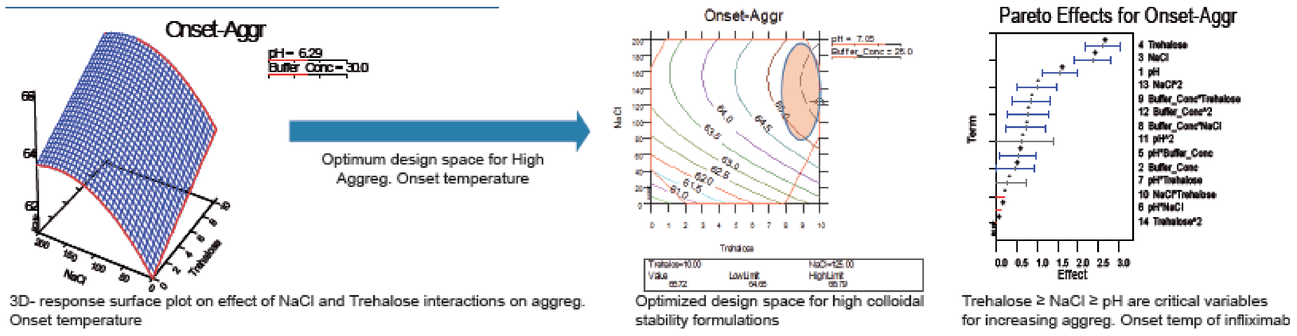


Fig. 6 : Infliximab (Remicade®) aggregation Decrease with increasing hp molarities

Infliximab relative degree of aggregation decreased from 260 mAU in water (control) to 210 mAU (in 100 mM HP) and to 120 mAU (in 200 mM HP).



3D- response surface plot on effect of NaCl and Trehalose interactions on aggreg. Onset temperature

Optimized design space for high colloidal stability formulations

Trehalose ≥ NaCl ≥ pH are critical variables for increasing aggreg. Onset temp of infliximab

Fig. 4 : Infliximab (Remicade®) colloidal stability by Pareto and DOE analysis of *i*FORMULATE™ formulations

CONCLUSION

Preliminary results demonstrate that KLEPTOSE® HPB BioPharma hydroxypropyl-ß-cyclodextrin and KLEPTOSE® HP BioPharma hydroxypropyl-ß-cyclodextrin at high molarity (200 mM) are efficient tools in modulating Infliximab relative degree of aggregation.

REFERENCES

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