T1530-09-56

Points to Consider in the Development of Activity and Potency Assays for Enzyme Biotherapeutics

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PURPOSE

The ability of enzymes to remain active outside their source organism makes them an ideal candidate for biotherapeutics. Some examples include recombinant enzymes, in-process enzymes, and gene therapies intended to produce or replace enzymes to directly address a disease. The activity of these enzymes can be measured in many ways including absolute activity, specific activity, relative activity, or kinetic activity.

The purpose of the work presented is to highlight various assay strategies to quantify enzymatic activity in ancillary material and drug products by using science-based and risk-based strategies.

OBJECTIVES

- Demonstrate specific activity for biopharmaceutical products using clot lysis time kinetic assay.
- Demonstrate the feasibility of using an off-the shelf commercial activity kit to develop an enzymatic activity assay for ancillary products used in biopharmaceutical manufacturing.

METHODS

Detection Chemistry: Clot Lysis Time using Recombinant human tissue Plasminogen Activator (rt-PA) as positive control , Trypsin Activity Colorimetric Assay Kit (MAK290).

Imaging Platform: BioTek Synergy NEO2 (Agilent Technologies, Santa Clara, CA

Data Analysis: Gen 5 Secure version 3.02 (Agilent Technologies), Solver Spreadsheet (Microsoft Excel).

Figure 1(a),(b), and (c): Clot lysis kinetic plots for IRP, DS and DP at different levels of concentration. Clot formation is linked to the formation of insoluble fibrin which causes turbidity and increased absorbance. The kinetics of clot formation are very rapid (a few seconds). Then a plateau is observed which indicates the formation of a stable clot followed by the lysis of the clot that starts with the progressive dissolution of the fibrin and therefore a decrease in turbidity and absorbance until to reach the baseline.

Figure 2(a),(b) and (c): Linear regression plots for IRP, DS and DP at different levels of concentration. The maximum absorbance on the kinetic curve is divided by 2. The time at which we observe the first absorbance value lower than Amax/2 is considered as clot lysis time. Then a graph of log(t) versus log(C) is plotted and a linear regression is performed.

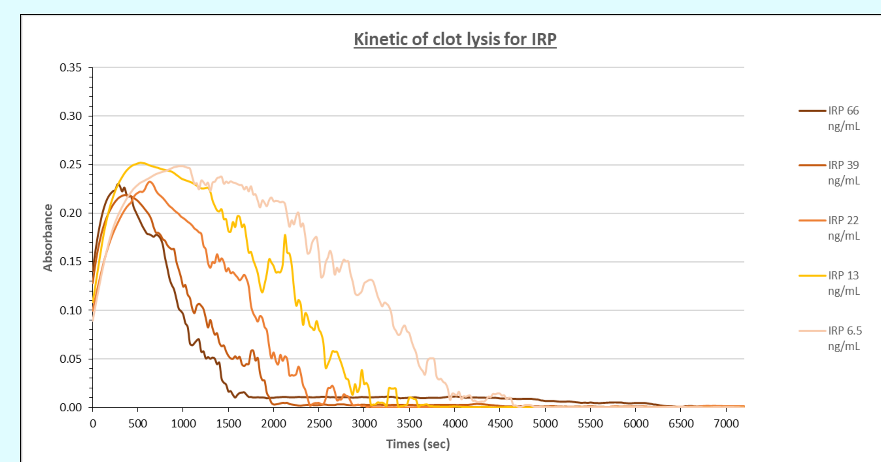


Figure 1(a)

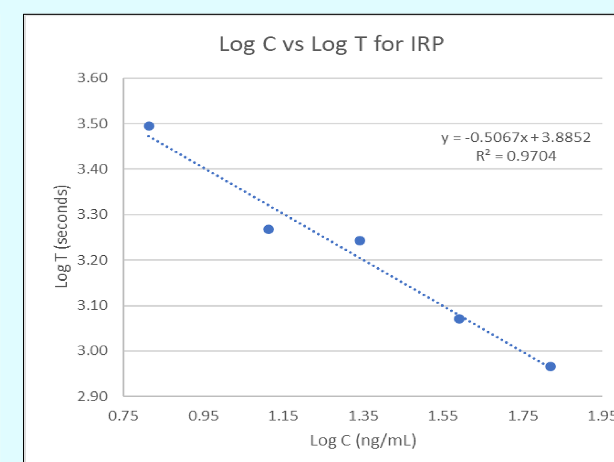


Figure 2(a)

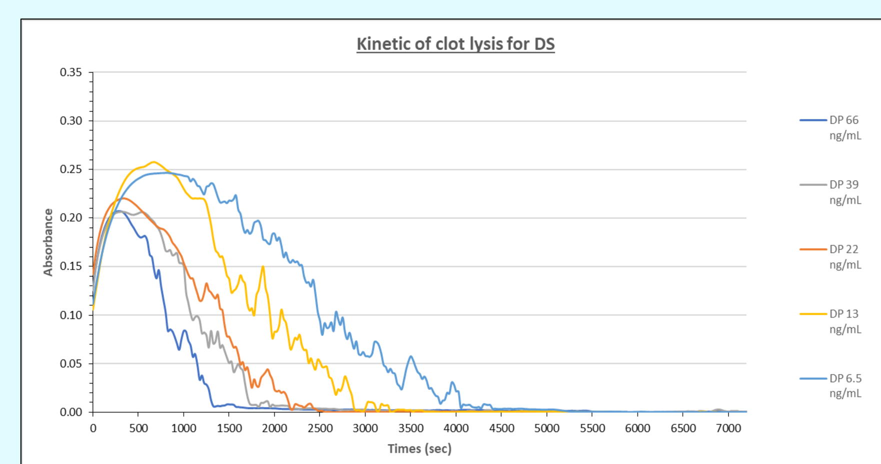


Figure 1(b)

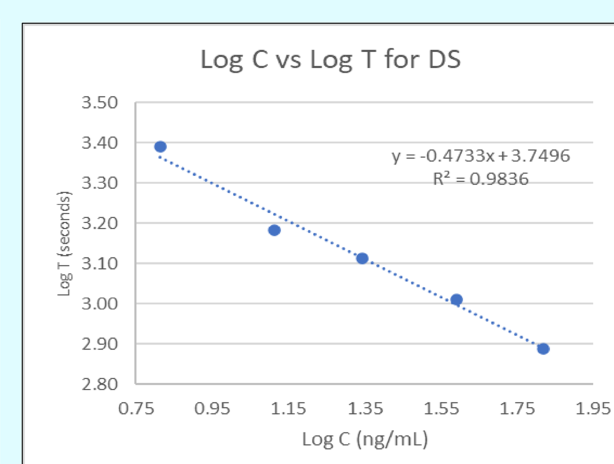


Figure 2(b)

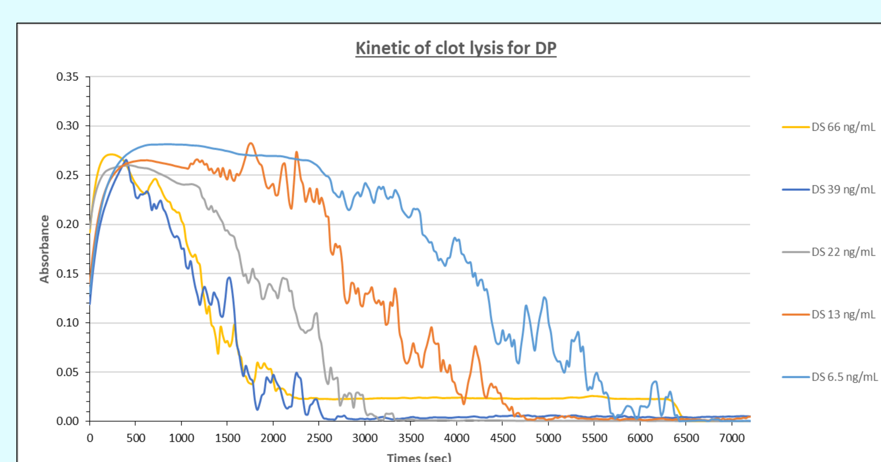


Figure 1(c)

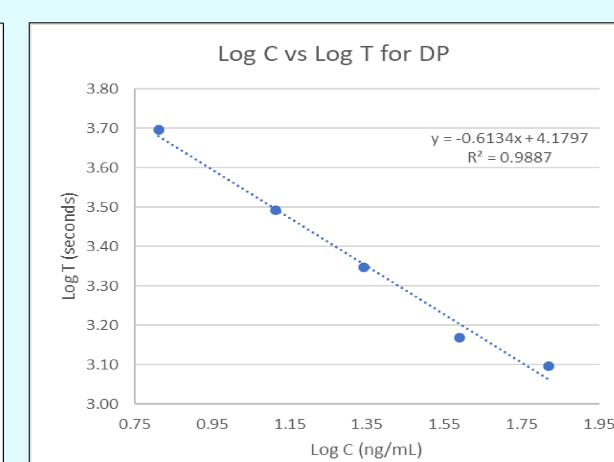


Figure 2(c)

Figure 2: Kinetic plot of trypsin activity for immobilized enzyme and trypsin reference material. Trypsin activity derived from the samples appear to plateau within 2 minutes of the reaction.

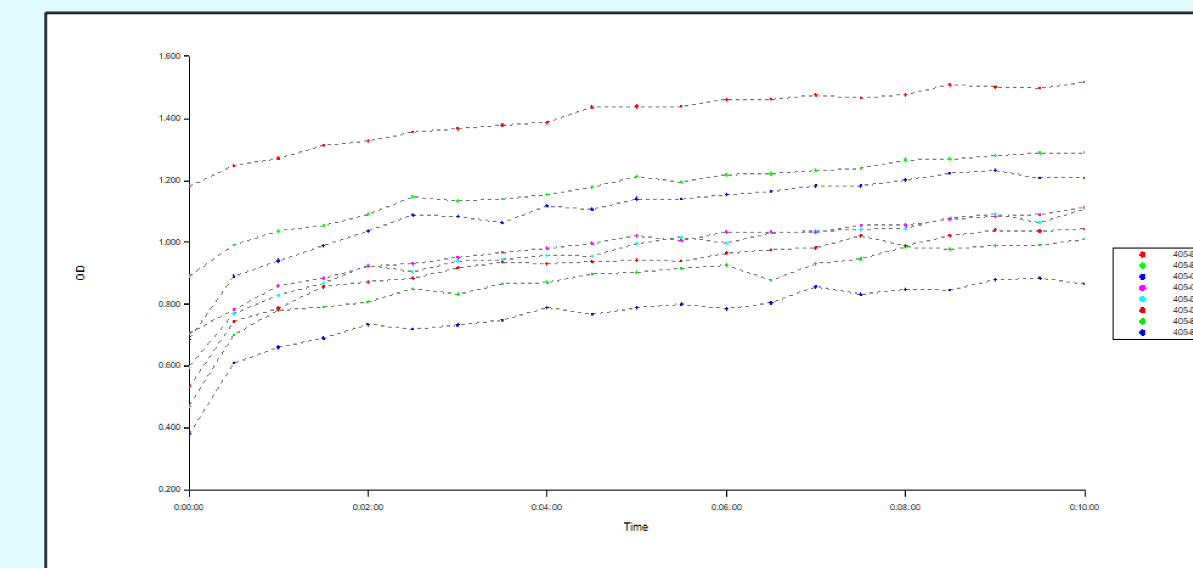


Figure 3: 5-point dilutional linearity was demonstrated by a linear regression between wet bead weight and the mean back-calculated p-NA concentration.

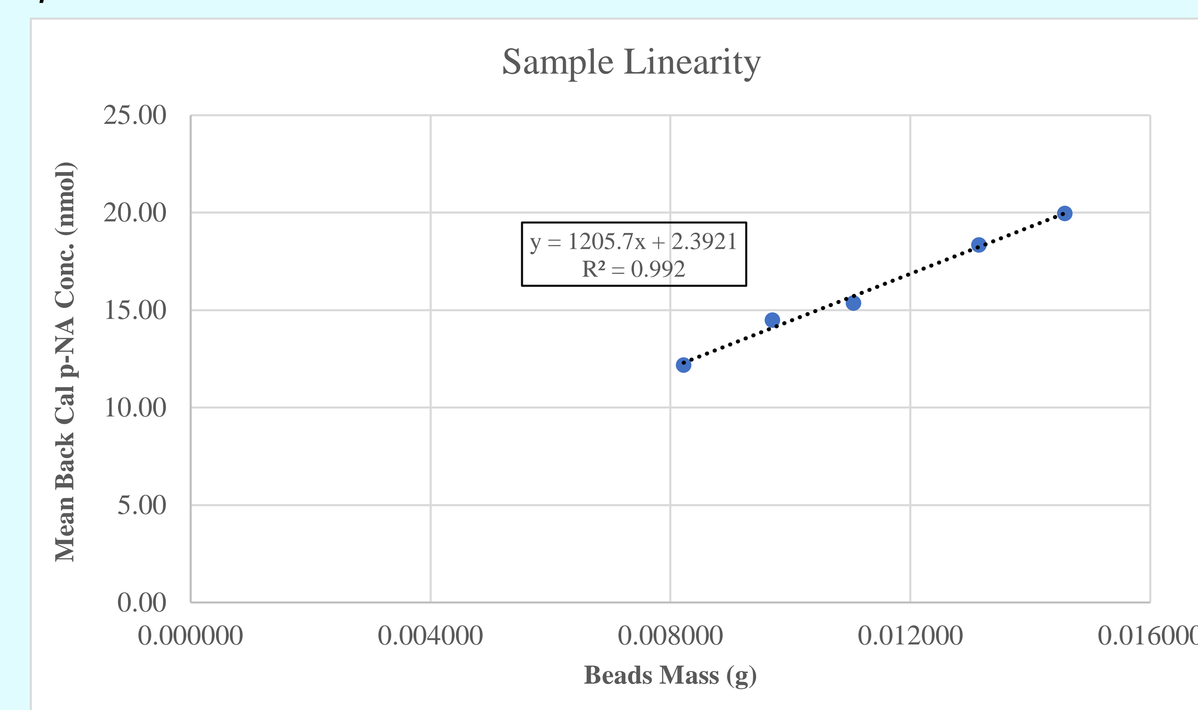
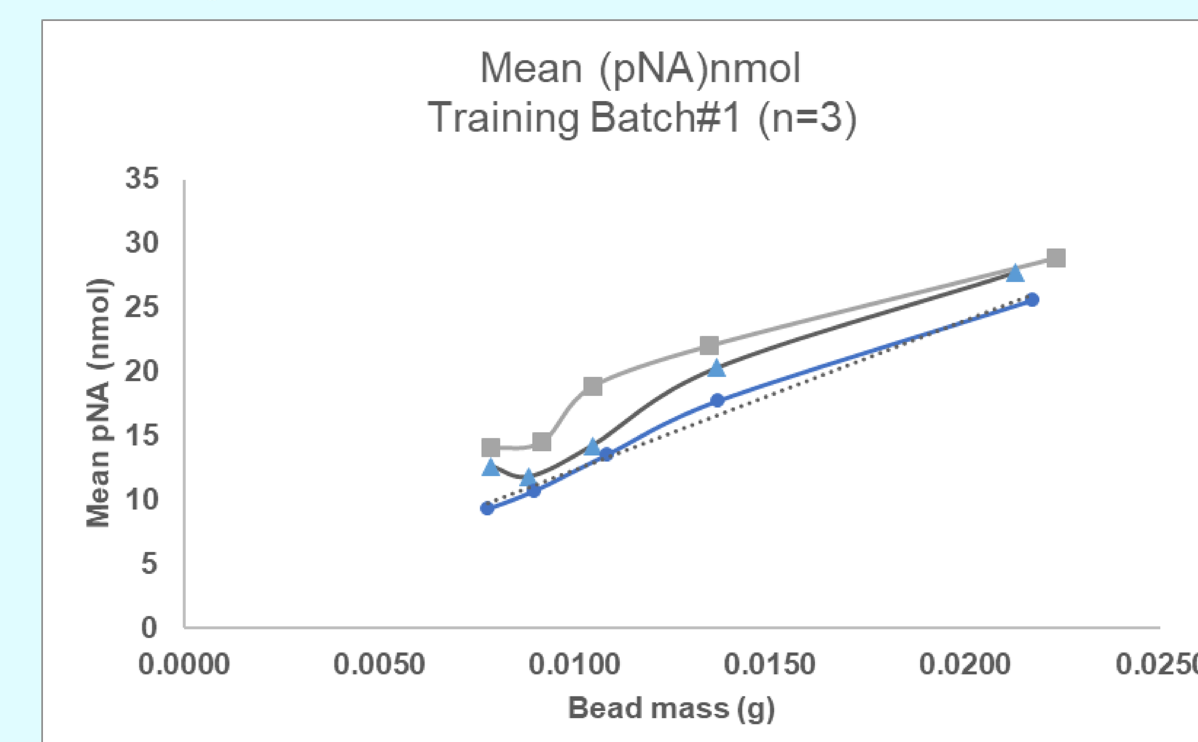


Figure 4: Repeatability was demonstrated for a 5-point dilutional linearity was demonstrated by a linear regression between wet bead weight and the mean back-calculated p-NA concentration.



CONCLUSIONS

1. Comparative analysis of the rate constants for each enzyme – WHO reference, drug substance and drug product were different in their time to clot lysis. The use of specific activity determination provided insights into kinetic profile of the enzymes.
2. Gravimetric measurement of trypsin beads allowed an accurate estimation of Trypsin activity. The samples exhibited 90-112% of the nominal value, in addition, the repeatability and intermediate precision was ≤ 20% (%CV of the mean p-NA nmol/g)
3. Both in-house and commercial enzymatic assays may be leveraged to quantify enzymatic activity for biopharmaceutics.
4. Enzyme activity is a critical quality attribute of many biotherapeutics with enzymatic activity as a component of their MOA. The insights gained from this research contribute to enhancing the rigor and reliability of use for drug products and ancillary materials in biotherapeutic production processes.

REFERENCES

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