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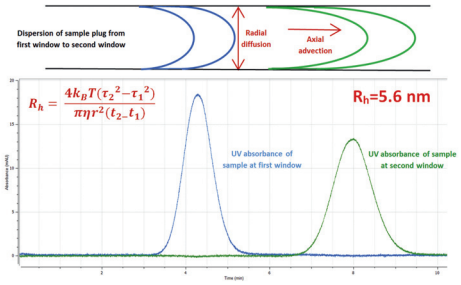
TAYLOR DISPERSION ANALYSIS – AN EMERGING TECHNIQUE FOR CHARACTERISING PROTEIN STABILITY: INSULIN AS A CASE STUDY

Introduction

As an important biotherapeutic used in the treatment of Diabetes Mellitus, Insulin provides an interesting case study with which to review the use of Taylor Dispersion Analysis (TDA) as a technique for characterising protein stability. Changes to Insulin's environment can cause changes to the protein itself, for example, Insulin can be found in a number of different oligomeric states under different formulation conditions.

Theory

Taylor Dispersion Analysis is a mass weighted sizing technique, which can be used to determine the hydrodynamic size of solutes by monitoring their dispersion whilst travelling through a capillary. TDA combines pressure driven convection with diffusive mixing in the radial direction to give the molecular diffusion coefficient and, in turn, hydrodynamic size. The larger the solute, the smaller the diffusion coefficient and therefore the greater the dispersion whilst travelling through a capillary. This theory can be applied to a wide range of solutes, from proteins, to peptides, to small molecules.



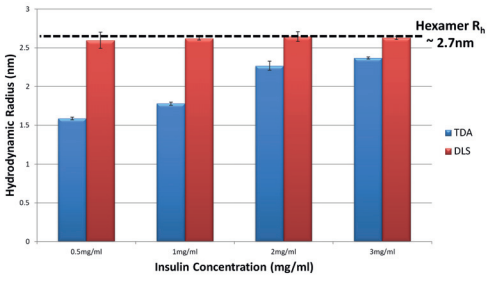
Above: Parabolic flow of a sample plug in a capillary showing dispersion from window 1 (blue) to window 2 (green)

Below: Taylorgram of 10mg/mL IgG in PBS

R_h = Hydrodynamic radius, K_B = Boltzman Constant, r = Capillary radius, T = Temperature, η = Viscosity of solution, t_1 and t_2 = time at respective peaks, τ_1 and τ_2 = Standard deviations of Gaussian distributions at respective peaks

Effect of protein concentration on oligomeric state

Insulin oligomers have been shown to dissociate at low protein concentrations[1]. This is key to allow the protein to perform its role within the body; hexameric Insulin is the more stable form used to store the protein within cells, whereas monomeric Insulin is the active form that binds to Insulin receptors. Identifying the oligomeric state of Insulin in a specific formulation is therefore important in predicting the stability of the formulation. Hexamers are formed through the self association of Insulin monomers into dimers, which combine to form hexamers. Changes in Insulin's oligomeric state can be observed as a change in size:



Sizes observed by DLS (red) and TDA (blue) at different Insulin concentrations

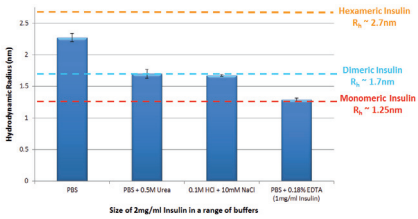
The size observed by TDA decreases as the concentration of Insulin decreases, indicating that the proportion of hexameric insulin is decreasing with concentration. This trend is also observed by DLS, but it is not as pronounced as the technique is more sensitive to the larger hexamers present within the sample.

References

1. A.M. GUALANDI-SIGNORINI, G. GIORGI, "Insulin formulations – a review" European Review for Medical and Pharmacological Sciences 5: 73-83 2001
2. Atta Ahmad, Ian S. Millett, Sebastian Doniach, Vladimir N. Uversky, Anthony L. Fink, "Stimulation of Insulin Fibrillation by Urea-induced Intermediates" Journal of Biological Chemistry 279: 14999-15013, 2004
3. Michael M. Varughese, Jay Newman, "Inhibitory Effects of Arginine on the Aggregation of Bovine Insulin" Journal of Biophysics Volume 2012, Article ID 434289, 7 pages, 2012

Effect of formulation buffer on oligomeric state

Addition of excipients to formulations can affect the protein itself; Insulin's oligomeric state is altered by the addition of excipients as well as changes to the buffer pH. TDA can be used to monitor these changes: (The changes observed by TDA are in good agreement with literature values[2])

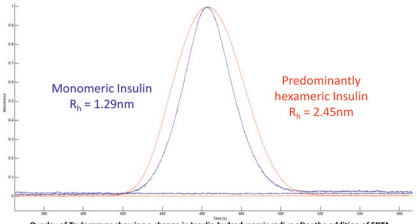


Size of 2mg/ml Insulin in a range of buffers

Understanding structural changes upon cofactor removal

Insulin is hexameric in the presence of Zinc. Removal of Zinc ions by EDTA therefore causes a shift in oligomeric state to the monomeric form. Using TDA, this is observed as a change in size.

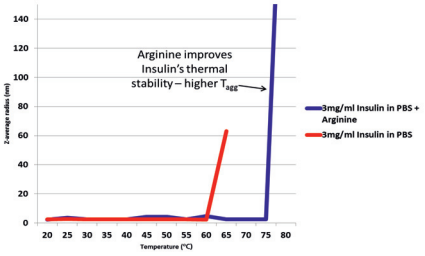
The Taylorgram of hexameric insulin has a wider absorbance peak than the smaller, monomeric Insulin. This is due to the differences in size of the two Insulin samples; the hexamer is larger and therefore disperses to a greater extent whilst travelling through a capillary, giving a wider peak in the Taylorgram.



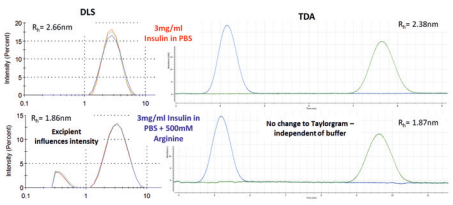
Overlay of Taylorgrams showing a change in Insulin hydrodynamic radius after the addition of EDTA (absorbances normalised to 1)

Improving Insulin's thermal stability through the addition of Arginine

Protein stability is one of the primary concerns during biopharmaceutical development, as it negatively impacts upon immunogenicity and potency of the biotherapeutic. Excipients are added to formulations to improve stability, but can provide complications during analysis of the protein. For example, Arginine is an excipient added to many formulations and it has been shown to prevent Insulin aggregation[3]. A thermal trend was performed to demonstrate Arginine's capacity to improve Insulin's thermal stability:



Excipients can interfere with analysis of the protein, particularly when the excipient is very large and similar in size to the protein. However, TDA is able to selectively monitor the molecule of interest, independent of formulation components.



Left – DLS plots of Intensity vs Size of 3mg/ml Insulin in different buffers

Right – Taylorgrams of 3mg/ml Insulin in different buffers