An atomic force microscopy based method for biopharmaceutical stability determination

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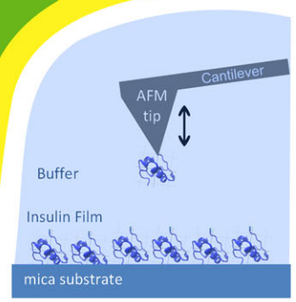
How Can We Improve Formulation Development?

Develop a simple technique for the rapid determination of biopharmaceutical stability using small quantities of active ingredient and formulation.

We propose that atomic force microscopy (AFM) based adhesion force measurements have potential in this application.

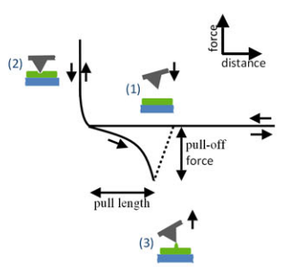
This approach could reduce the amount of resources required for testing in early formulation development, providing reliable biopharmaceutical stability information.

What Our Study Involves



We adsorbed bovine pancreatic insulin (BPI), onto a cleaved, clean mica surface (see AFM image) and used a silicon based AFM tip or an insulin functionalised tip to probe the adhesive interactions.

A typical AFM graph for a protein being mechanically stretched /unfolded is shown here with tip-sample force on the y-axis and approach distance on the x-axis.



(1) Approaching the surface there is no interaction force.
(no cantilever bending)

(2) In contact with the surface there is a repulsive force.
(upward cantilever bending)

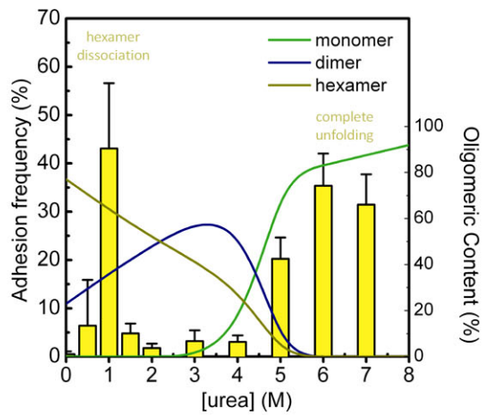
(3) On retraction from the surface an adhesive force may be present. A certain force and distance of retraction will be required to overcome this adhesion.
(downward cantilever bending)

AFM & AUC Combined

AFM

Each bar represents the average frequency of "adhesion events" over 25 different points on the insulin coated surface for each urea concentration.

$[D]^{50\%}$ = 4.9 ± 0.2 M
ΔG = 8.8 ± 0.2



AUC

Each line represents the monomer, dimer or hexamer content of an insulin solution at each urea concentration .

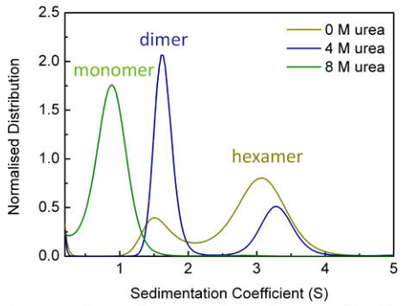
$[D]^{50\%}$ = 4.5 ± 0.1 M
ΔG = 23.5 ± 0.3

Using equations developed by Alan Fersht, both AFM and AUC give similar denaturation concentrations ($[D]^{50\%}$), however the calculated ΔG (stability) constants differ by a factor of 3. As the current AUC analysis assumes all monomers are unfolded, this is not a surprising result.

Similar analysis of the comparable work performed by Ahmad et al (2004) gave these values, which are more in line with the AFM results.

$[D]^{50\%}$ = 4.5 ± 0.1 M
ΔG = 4.8 ± 0.1

Analytical Ultracentrifugation (AUC)

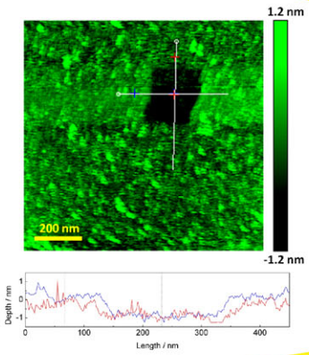


AUC was performed on 1 mg/ml solutions of insulin in citrate buffer at pH 7.4 and at different urea concentrations. This data shows clearly how the oligomeric state of insulin changes by addition of urea.

Insulin Surface

An AFM image of an insulin coated mica surface in a pH 7.4 buffer.

A 200 nm square area was scratched out using the AFM tip to measure the insulin layer thickness.

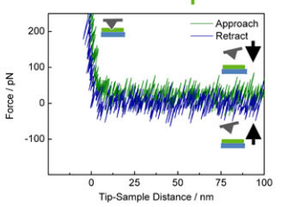


The lower graph shows cross-sectional traces indicating a layer thickness of 1 - 2 nm, thus a monolayer of coverage was concluded.

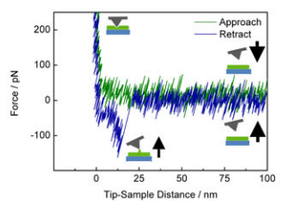
Conclusions

This AFM-based adhesion force technique that we are developing has been successfully used to provide reliable information about the concentration of urea required to unfold insulin. Comparison to AUC data and the work of Ahmad et al has shown that, with further refinement, this technique could be employed to obtain useful information about the stability of a given biopharmaceutical under formulation relevant conditions.

Explanation of Results



(A) an Insulin coated mica surface with no adhesion.



(B) an Insulin coated mica surface with adhesion.

We primarily consider two cases, A and B, of the AFM tip interacting with the insulin coated surface.

Considering case B as an "adhesion event" we examined the effected of increased denaturant concentration on the frequency of adhesion events.

The frequency was seen to be higher at various urea concentrations as shown by the bar graph in the "AFM and AUC Combined" box.

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