

The Challenges of Stabilisation in Protein Formulations

Dr. Jan Jezek

Arecor Ltd

Annual bioProcessUK Conference

Brighton, 26 – 27 November 2008

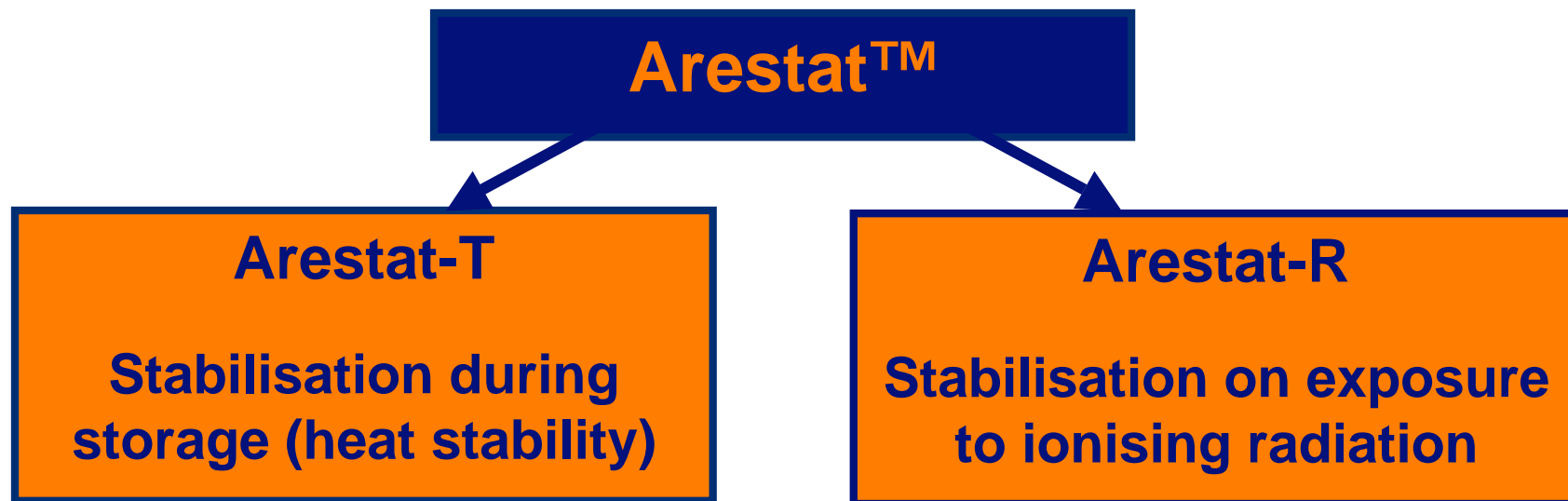




Bespoke solutions for protein stability

Close partnership with Biotech and Pharma companies

- Therapeutic applications
- Diagnostic applications
- Medical devices

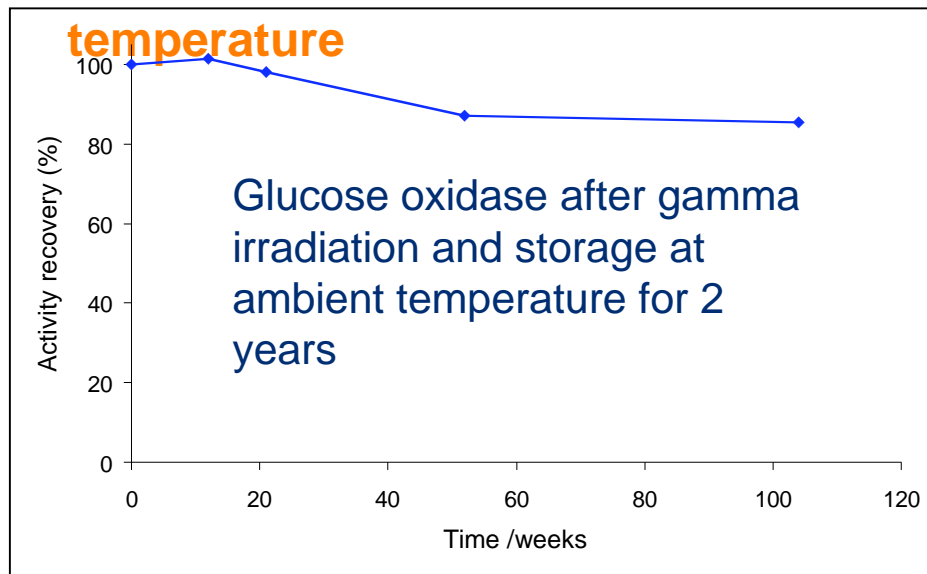


The Development of Arestat™

Arestat™ was developed to stabilize an active enzyme for use in a commercial wound care product

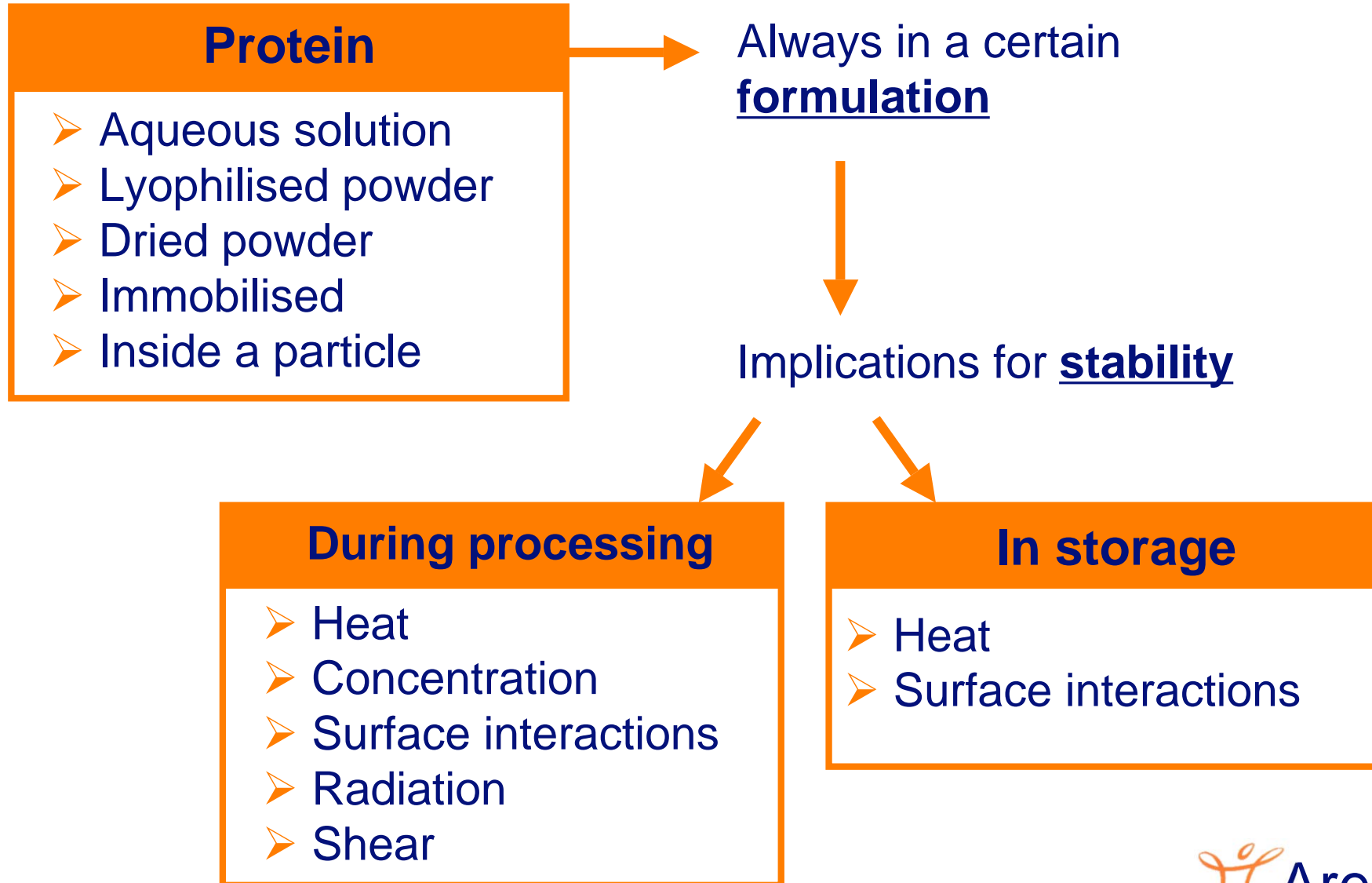
Needed to ensure stability:

- Through sterilization by ionizing radiation
- To maintain shelf life for storage at room



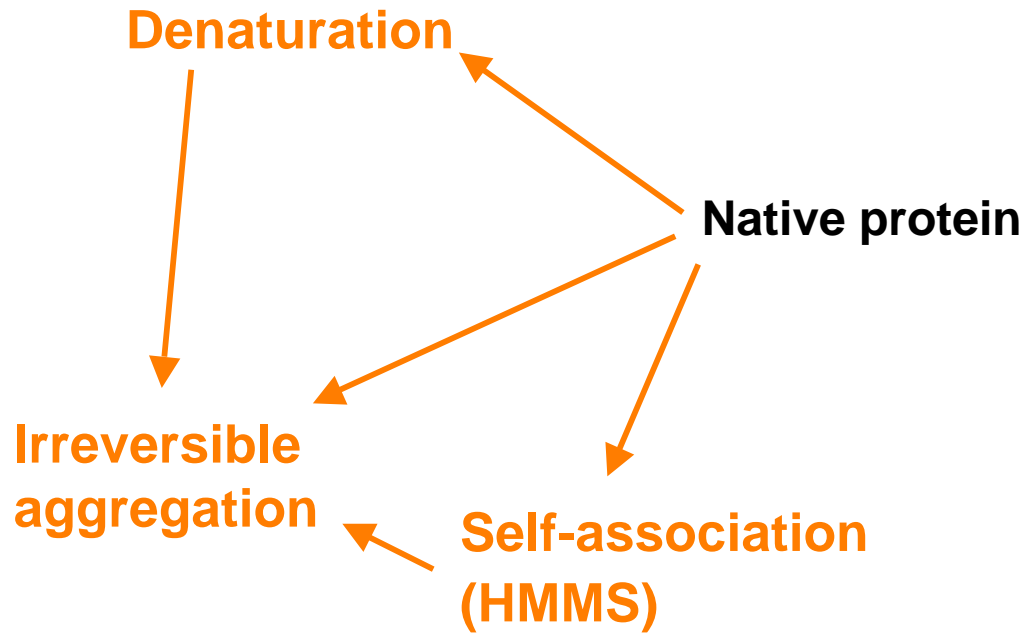
Oxyzyme has been approved by the MHRA as a Class 3 medical device and is marketed in the UK and Europe

Protein formulation – protein stability

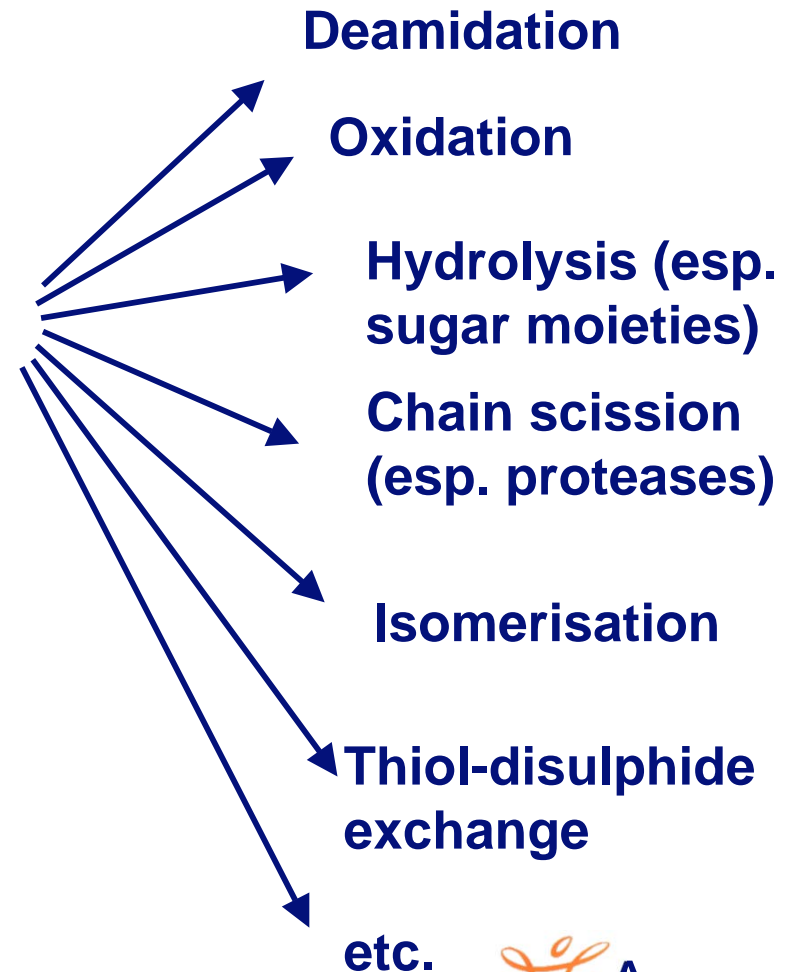


What can go wrong?

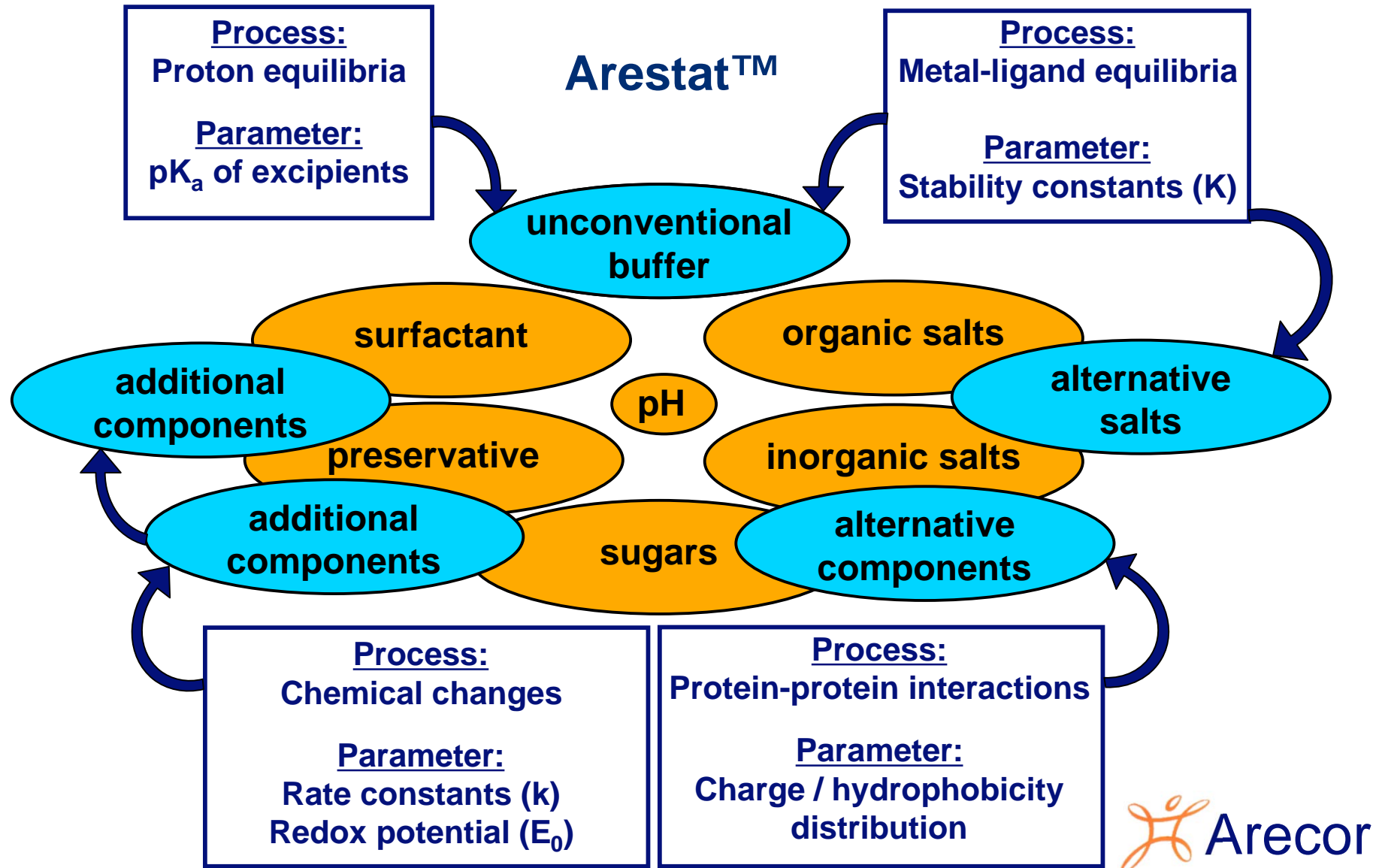
Physical instability



Chemical instability

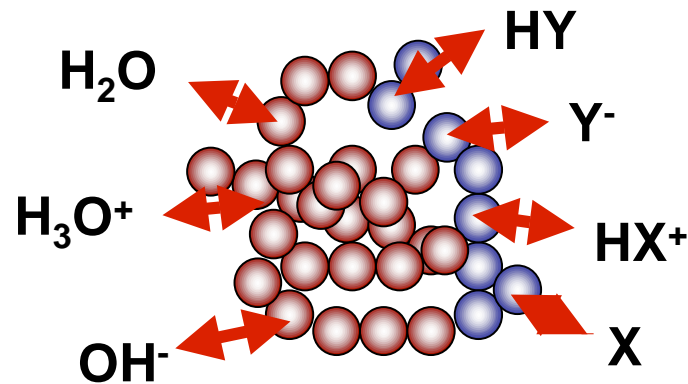


What can we do?

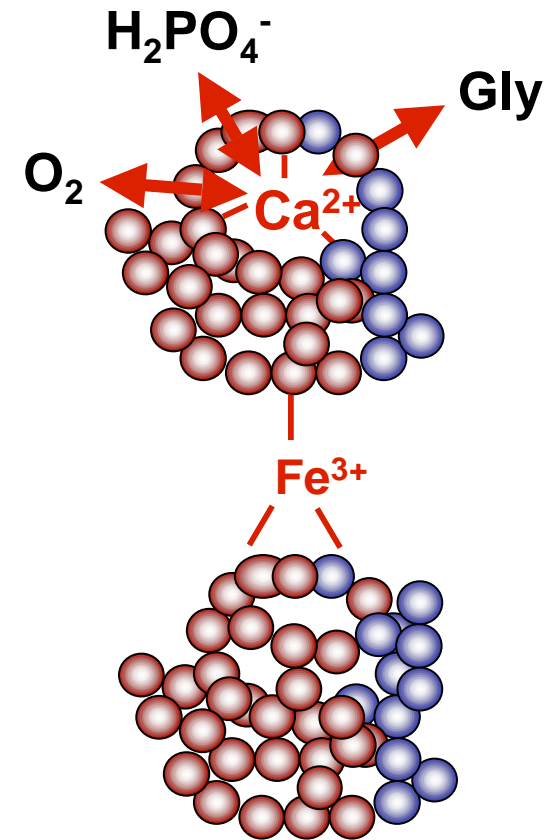


Reversible and irreversible interactions

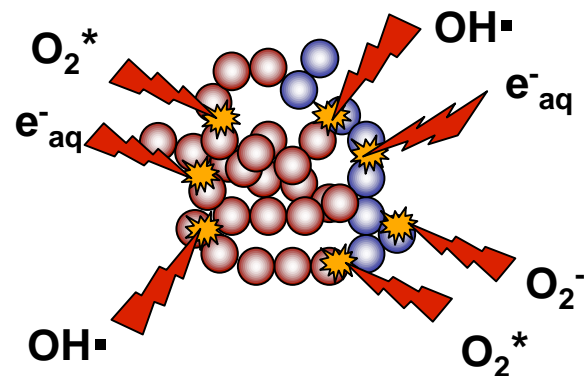
Proton exchange (pK_a)



Metal-ligand equilibria ($\log K$)



Irreversible changes (k , E^0)



Examples of formulation challenges

- High-concentration proteins
- Aqueous solutions instead of lyophilised powder
- Stability of complex systems (e.g. virus-like particles)
- Depot formulations
- Terminal sterilisation using ionising radiation

Terminal sterilisation by ionising radiation

Challenge:

Ionising radiation is a very convenient way of sterilisation (medical devices, diagnostic kits etc.)

Ionising radiation is very damaging to proteins

Protein	Current formulation	Arestat-R™
Glucose oxidase	8.2 %	96.2 %
Human growth hormone	34.4 %	97.8 %
Sandostatin	78.2 %	98.9 %
LL-37 growth factor	63.0%	99.0%
rPA (neat)*	24.0 %	94.0 %
rPA (on alum)*	12.3 %	93.4 %

Recovery based upon activity or RP-HPLC assays

* An ongoing project

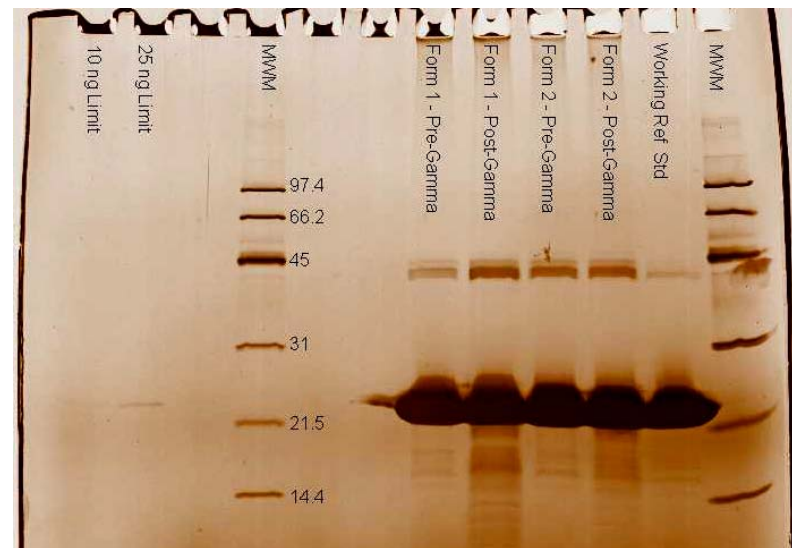
Terminal sterilisation by ionising radiation

Characterization of hGH (post-sterilization)

	hGH	Cyclic imides	Deamidated	Oxid (iD130)	Des-FP	Unk D	HMWS
Ref Standard	97.13%	0.55%	1.77%			0.58%	0.71%
Arestat-R pre-rad	97.09%	0.70%	1.66%	0.19%		0.36%	1.2%
Arestat-R post-rad	95.55%	1.74%	1.61%	0.90%	0.12%	0.30%	1.2%

Assessment by:

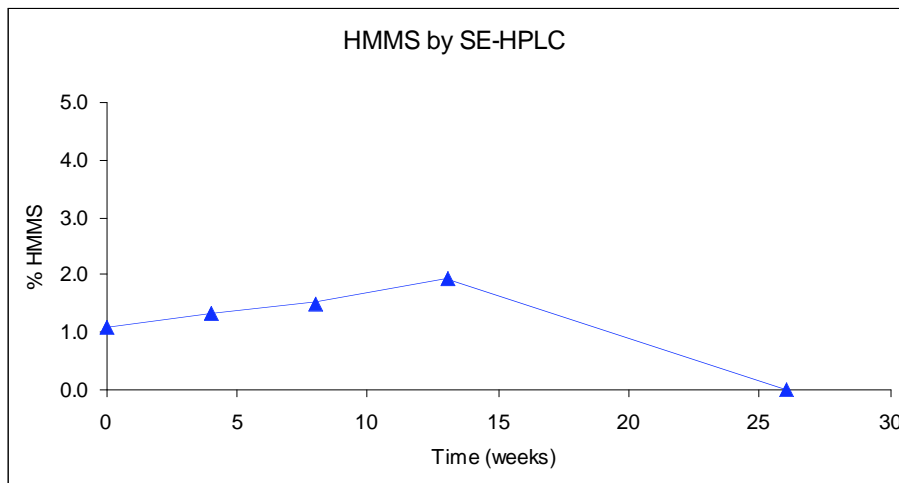
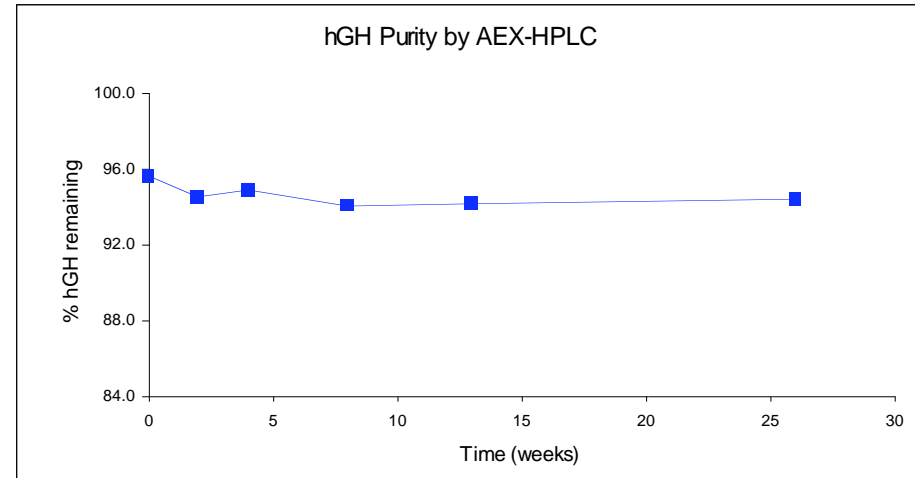
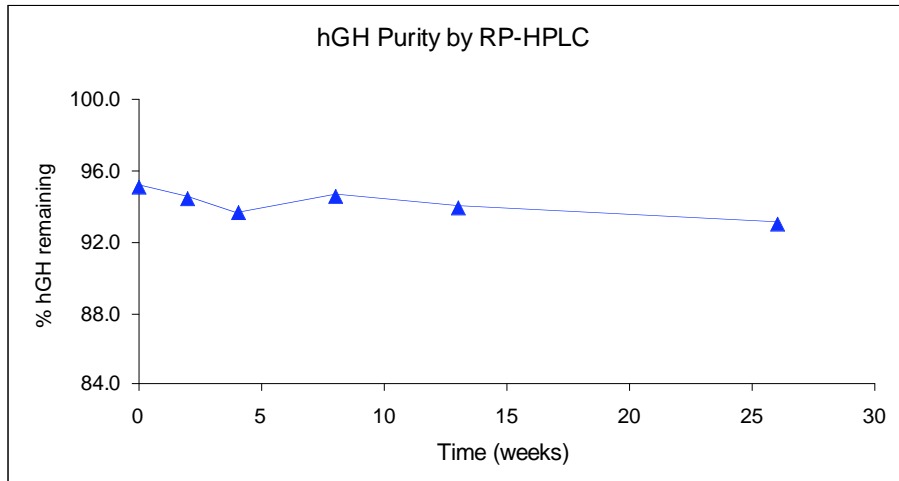
- RP-HPLC
- AEX-HPLC
- SEC
- SDS-PAGE
- Fluorescence



Terminal sterilisation by ionising radiation

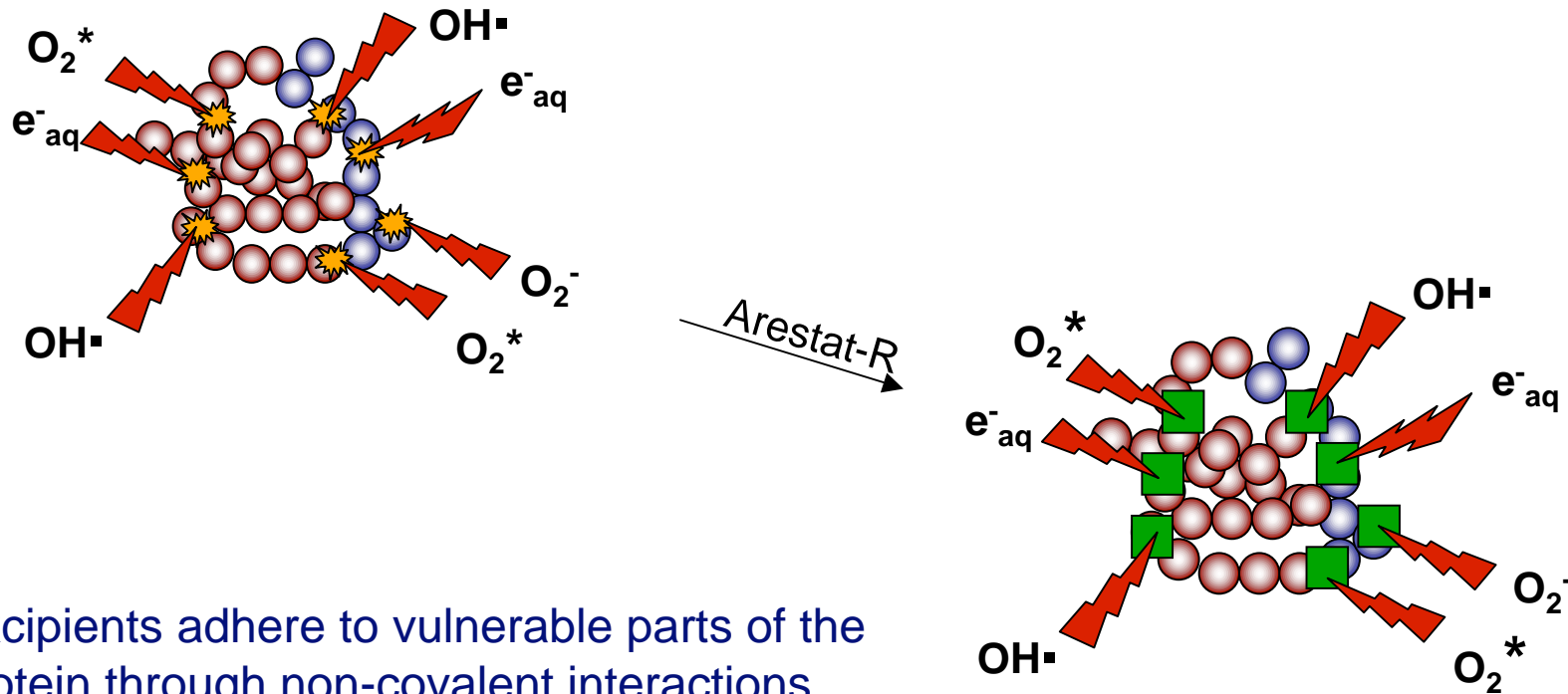
Latent effects of radiation often observed

Post-sterilisation stability of hGH (25 °C):



Terminal sterilization by ionising radiation (Arestat-R™)

Preventing irreversible reactions with high energy species during sterilisation



Excipients adhere to vulnerable parts of the protein through non-covalent interactions, and absorb the impact by the high energy species

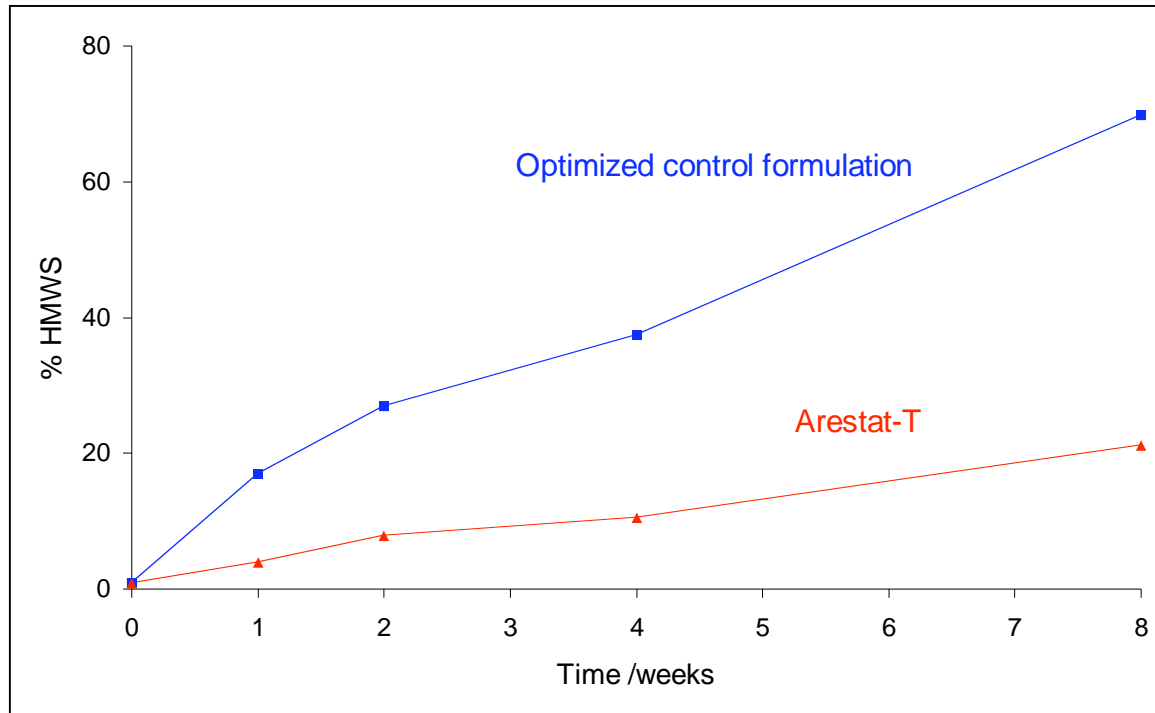
High concentration proteins

Challenge:

Aggregation and self-association are concentration-dependent processes

Viscosity is a key formulation parameter – may increase over time as self-association proceeds

Increasing need to formulate proteins at high concentration



Single-chain antibody
(10 mg/mL), 25 °C

Measured by SEC

Arestat-T formulation is exactly at the same pH as the control formulation

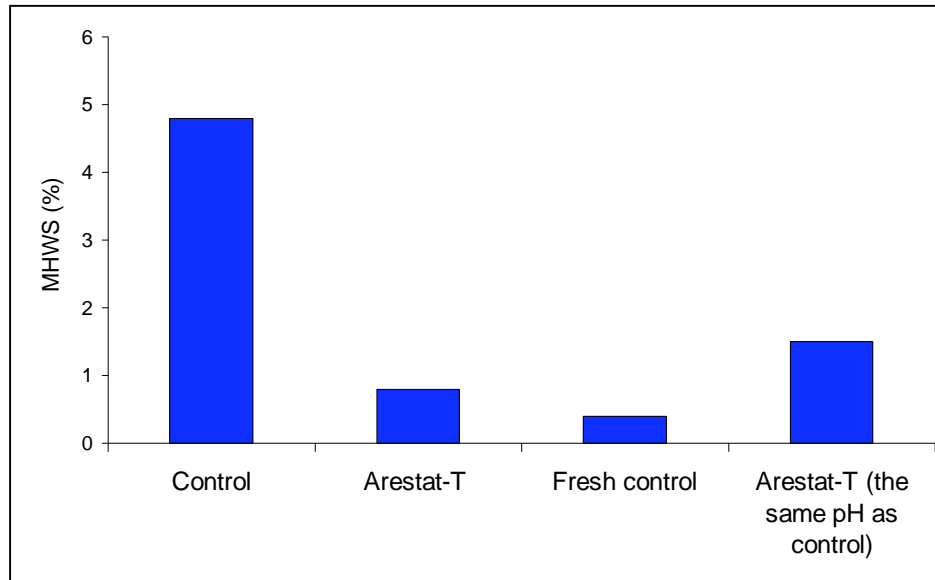
High concentration proteins

Monoclonal antibody at 40 °C – initial results

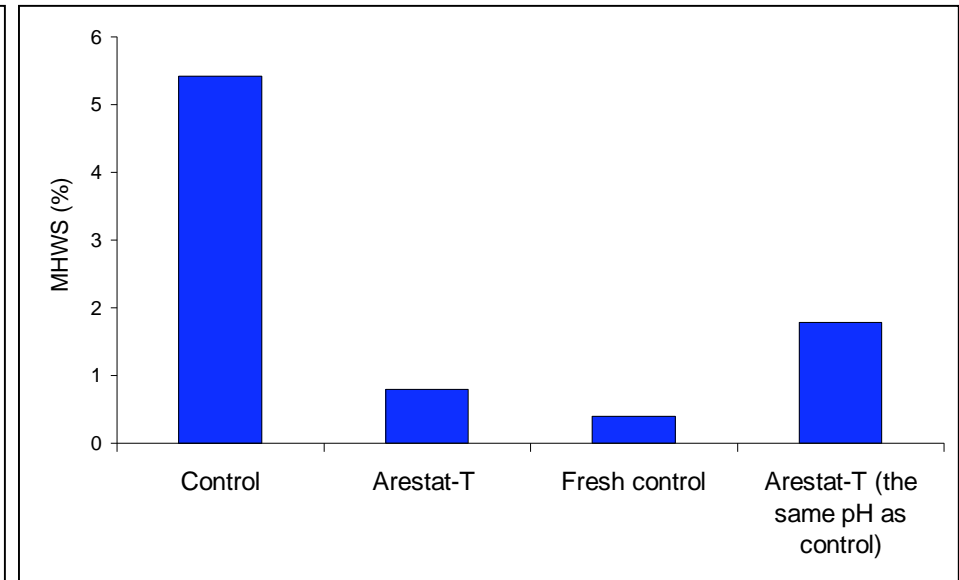
Soluble aggregates measured by SEC

Control = PBS, i.e. the original buffer

25 mg/mL, 4 weeks at 40 °C



100 mg/mL, 2 weeks at 40 °C

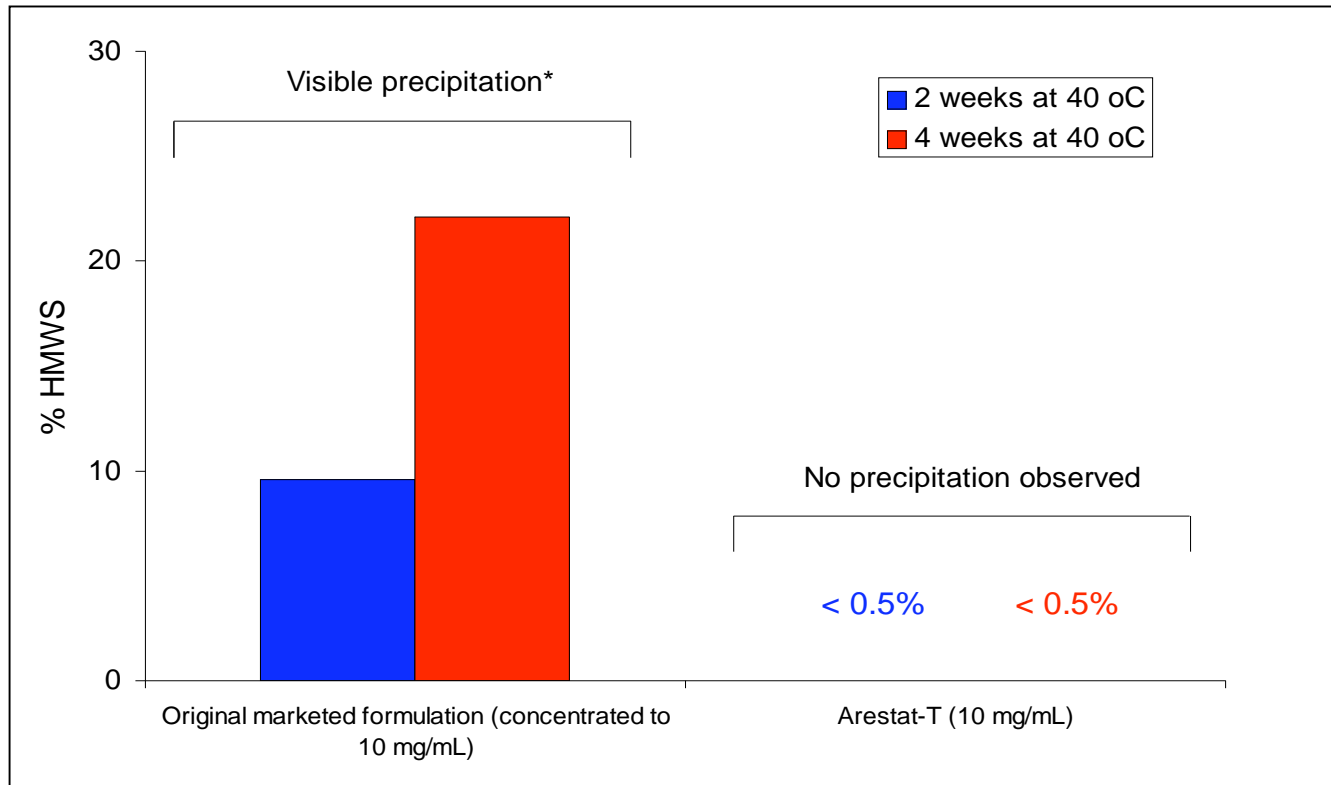


High concentration proteins

Human growth hormone (40 °C)

Increased tendency to aggregate at high concentration (especially >5mg/mL)

A convenient dosing form = substantial market advantage



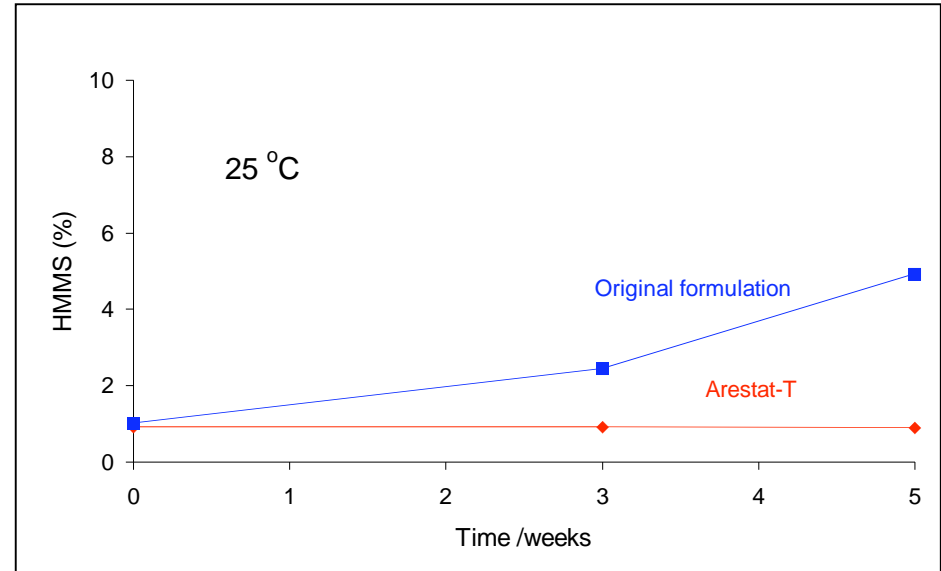
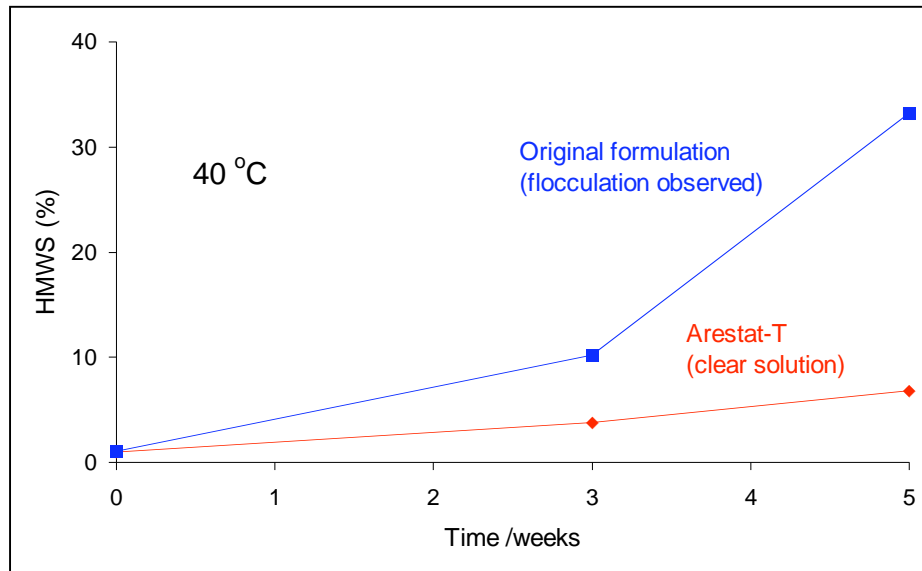
Measured using validated SEC method.

15 and 20 mg/mL also currently tested

High concentration proteins

Therapeutic enzyme

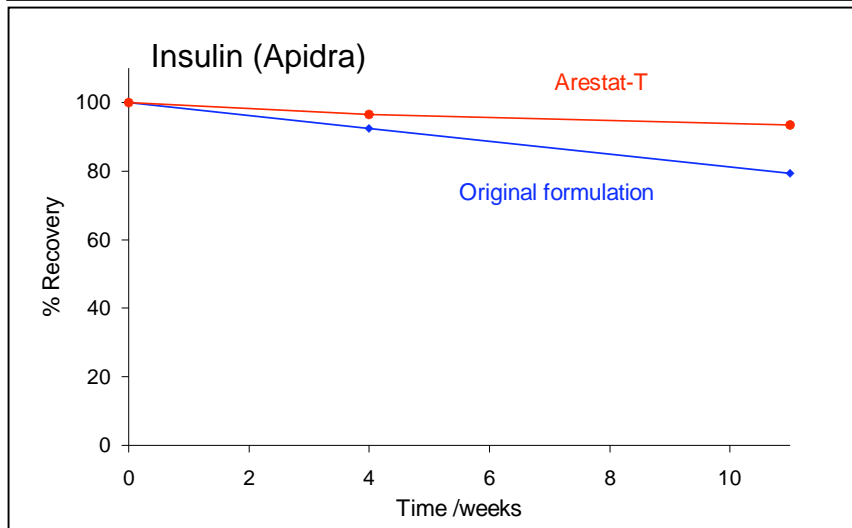
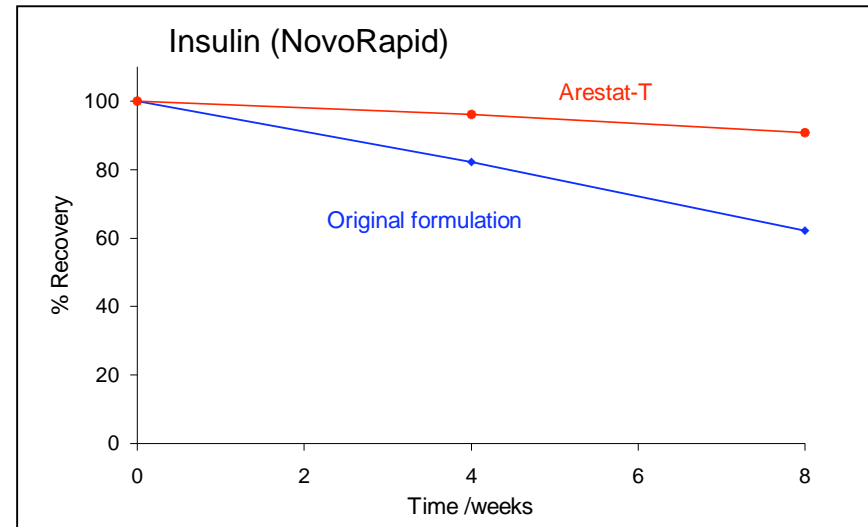
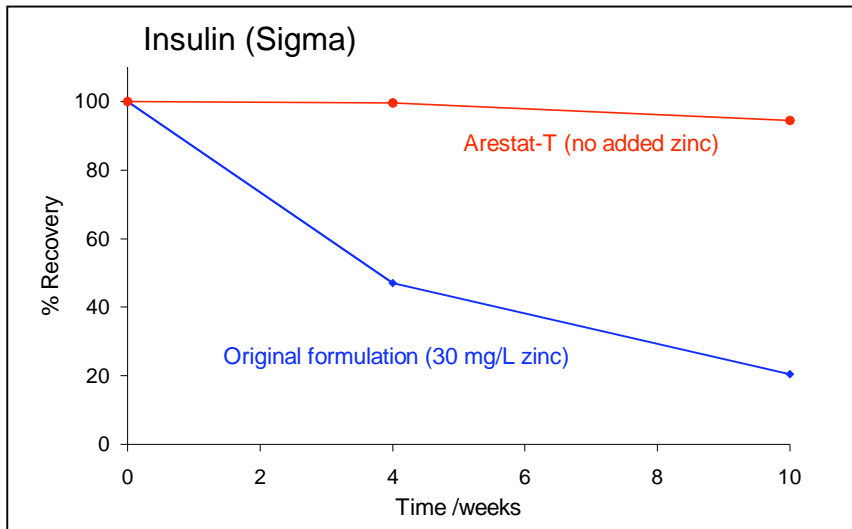
Current formulation is lyophilised; aggregation observed after reconstitution
Need to increase concentration from 5 mg/mL to 25 mg/mL



Measured using validated SEC method.

Depot formulations

Challenge: Sufficient stability at body temperature required



Recovery of native form of insulin following incubation at 40 °C

Assessed by Pharmacopoeia RP-HPLC method.

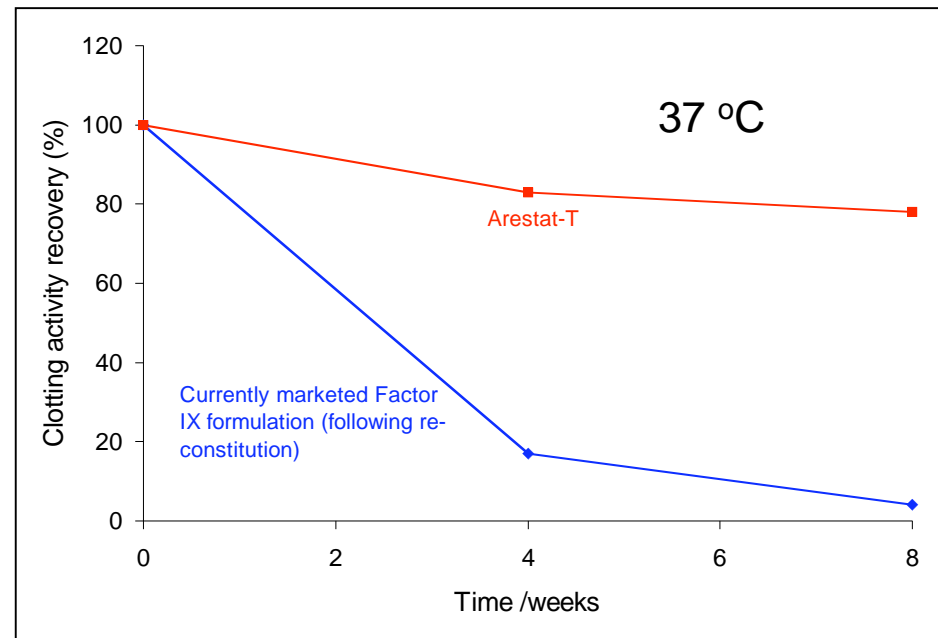
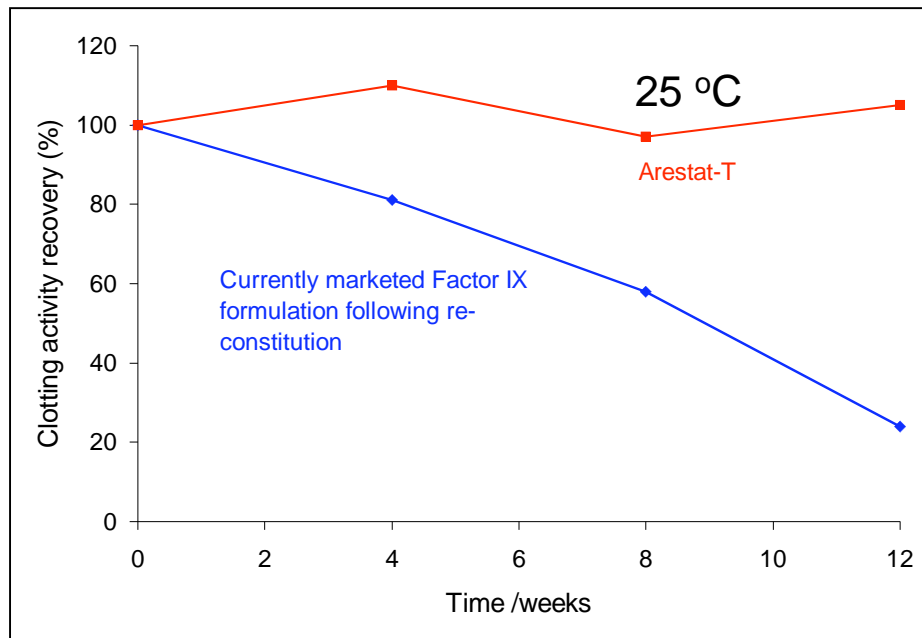
Apidra = monomeric form of insulin

Aqueous solutions instead of lyophilised powders

Challenge:

Factor IX is unstable in aqueous solution

Significant benefits of an aqueous preparations



The stability of FIX (100 IU/mL) in Arestat-T aqueous formulation and the marketed formulation in storage at 25 °C and 37 °C

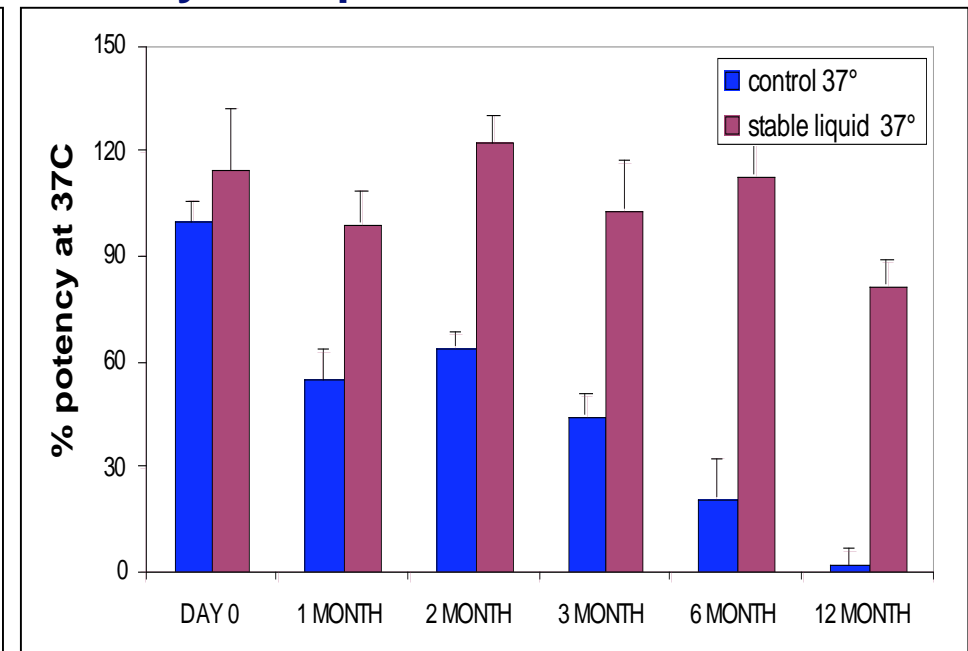
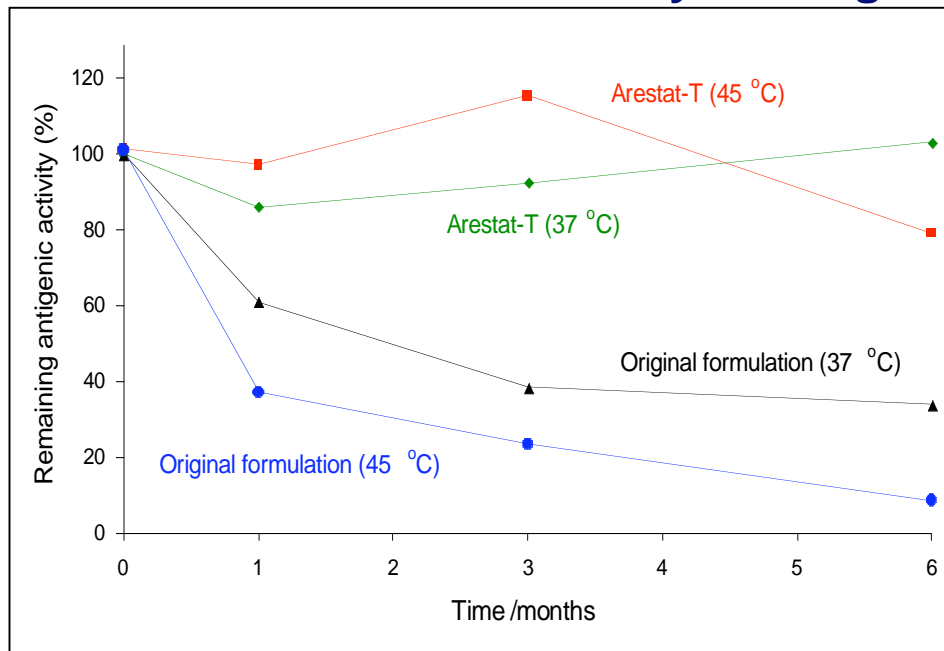
Stabilisation of virus-like particles

Hepatitis B vaccine

Currently marketed liquid formulation requires a cold chain

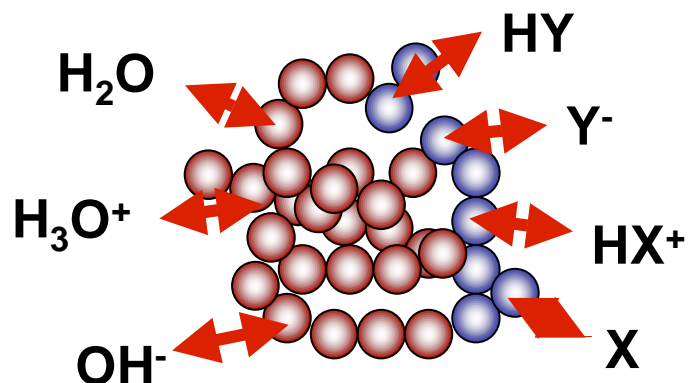
Arecor has developed a formulation of Hepatitis B vaccine stable at 45 °C for over one year – now in clinical development

Recovery of antigenic activity of Hepatitis B



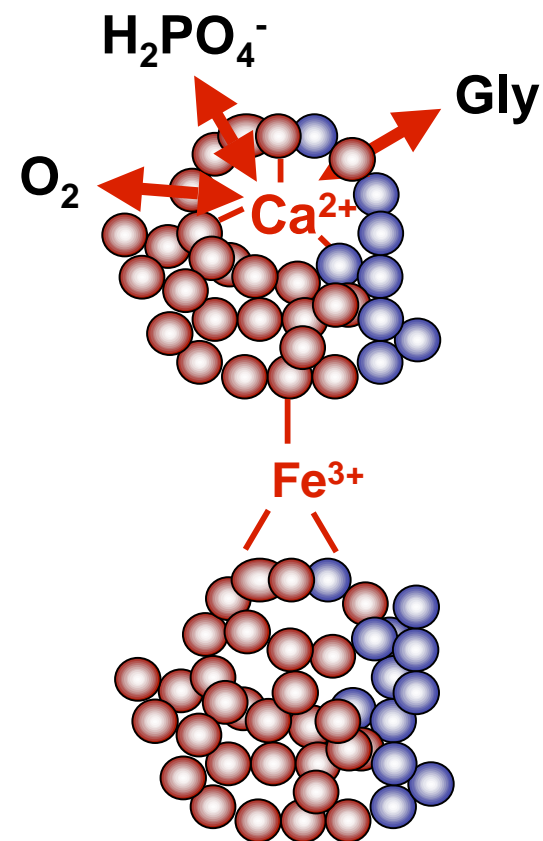
Equilibrium is a dynamic state

Proton exchange

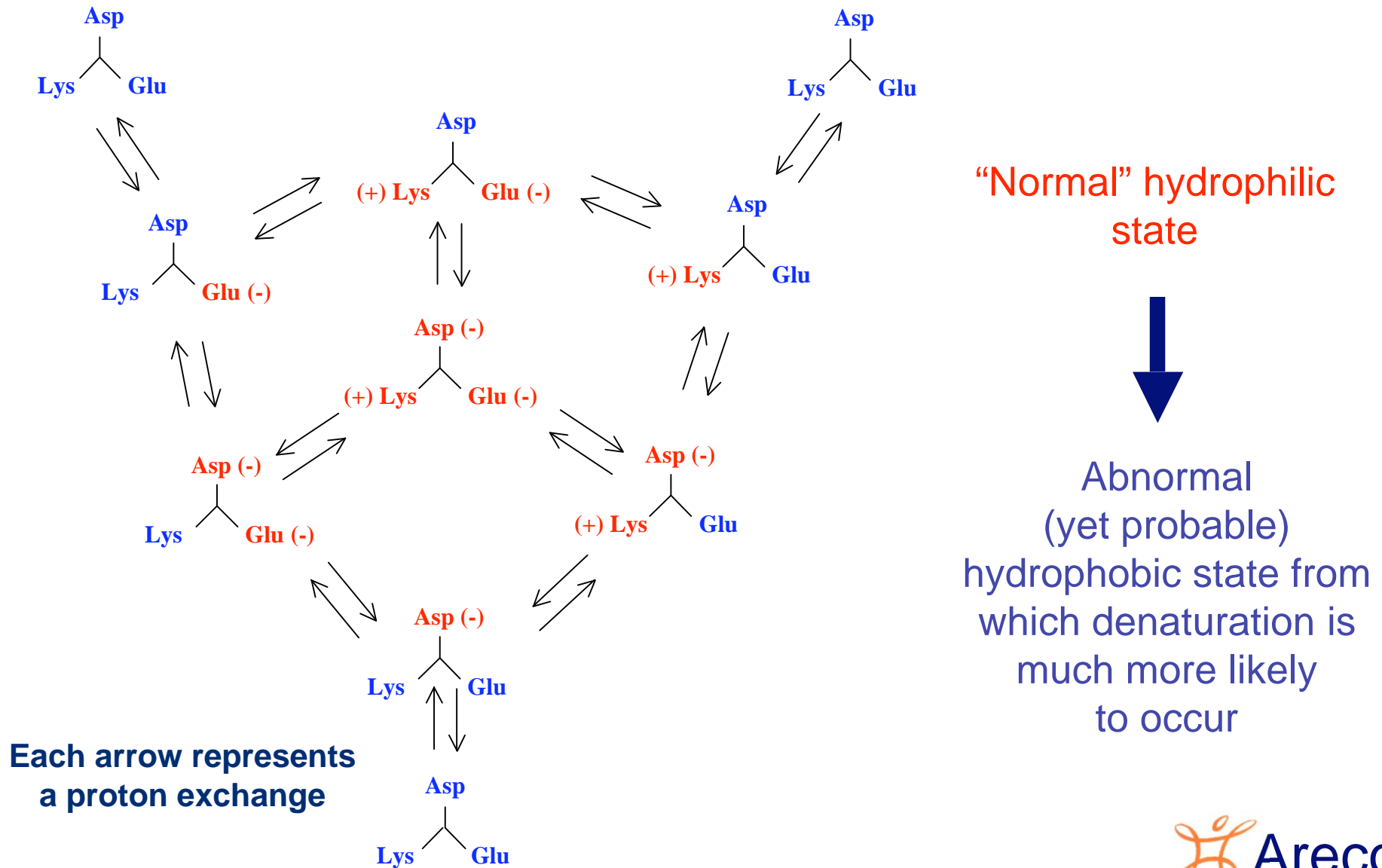


$$K = \frac{[A-B]}{[A][B]}$$

Metal-ligand equilibria



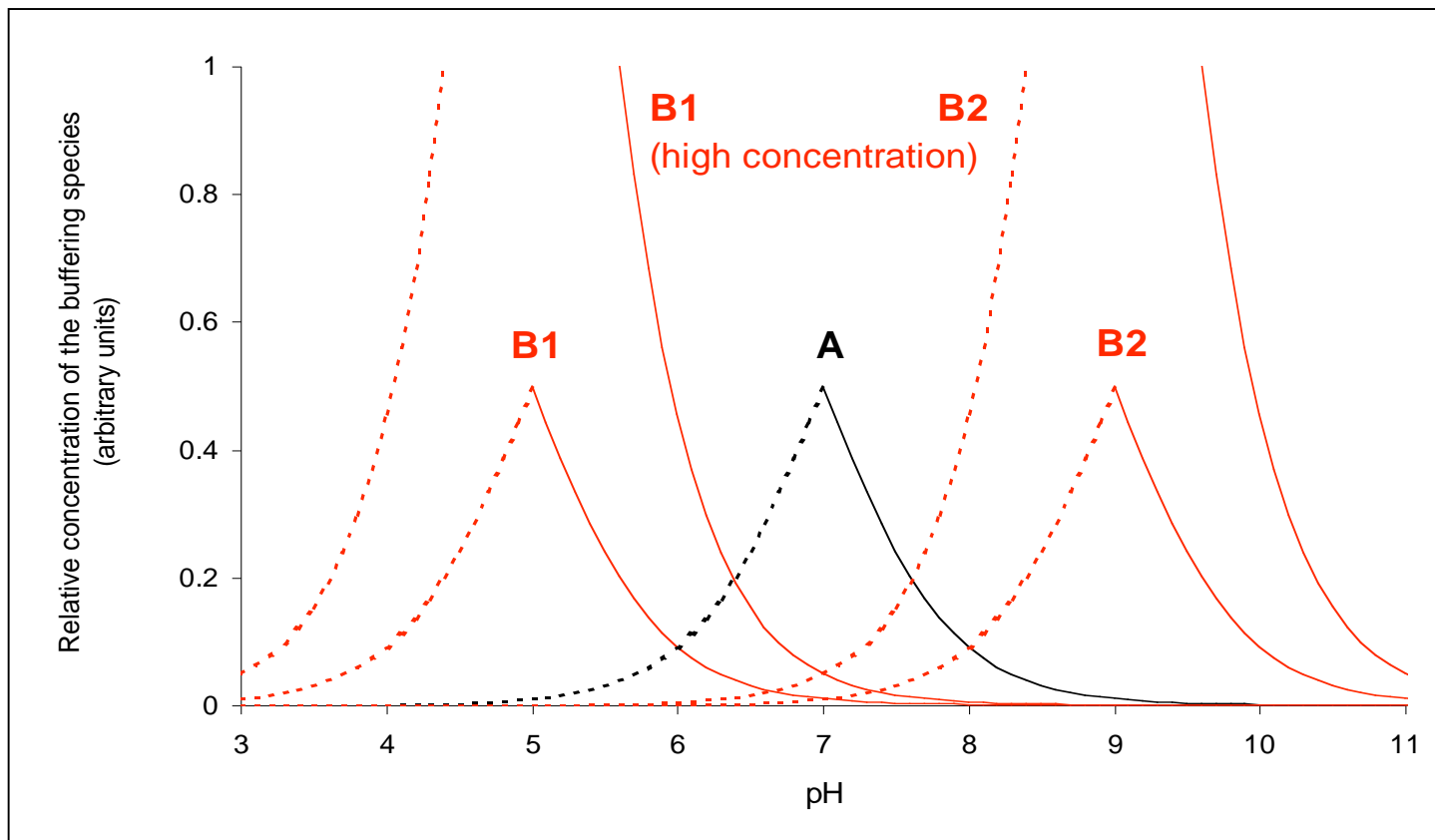
Protein adopts various states



Effect of the buffering system

Using buffers in an unconventional way

Maintenance of pH $[H_3O^+]$ through the application of “displaced buffers”



Representation of buffering efficiency of a conventional buffer (A) and two displaced buffers (B1, B2) to buffer a composition at pH 7: $pK_a(A) = 7$, $pK_a(B1) = 5$, $pK_a(B2) = 9$.

Metal – ligand interactions

A number of proteins rely on appropriate binding of a metal binding for their tertiary structure

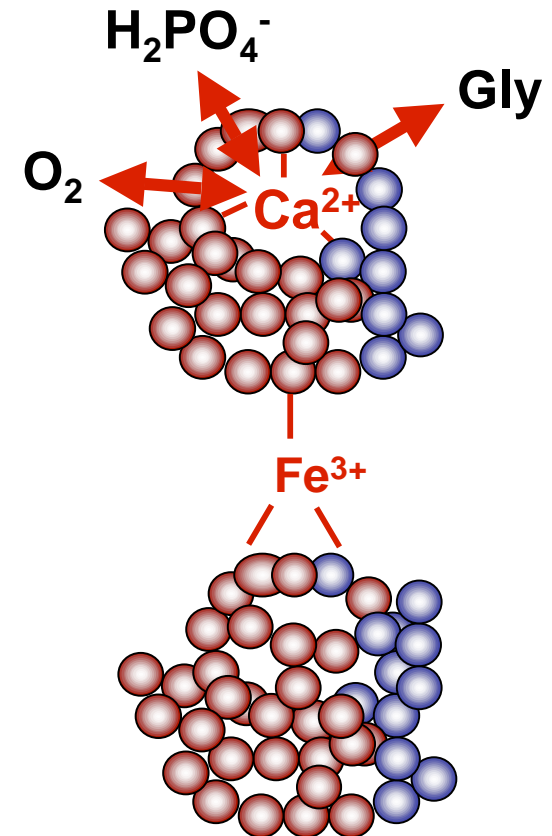
Metals also promote aggregation



$$K = \frac{[M-L]}{[M][L]}$$

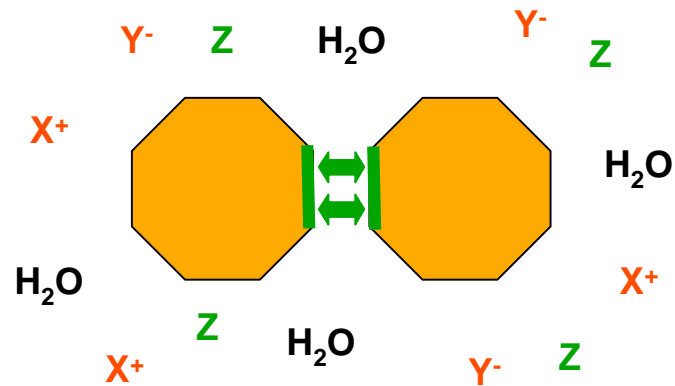
logK values (Ca²⁺ binding):

EDTA:	10.81
Citrate:	3.48
Glycylglycine:	1.21
Histidine:	1.24

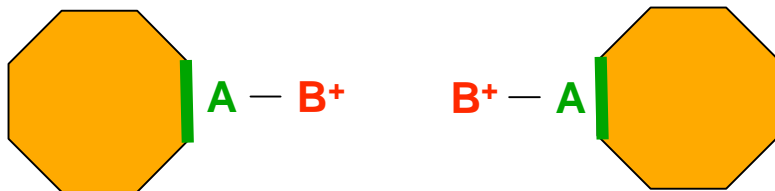
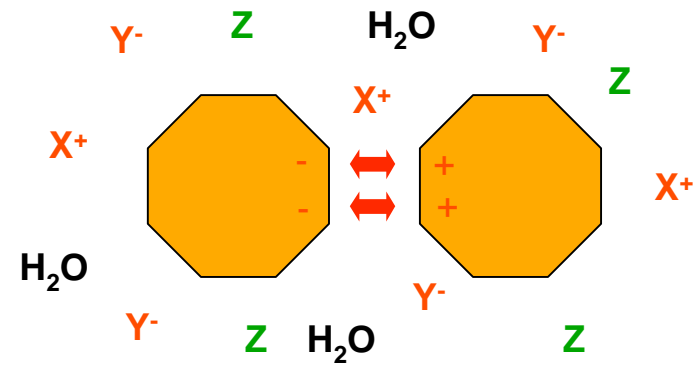


Self association

Hydrophobic interactions



Charge interactions



Charge / hydrophobicity

distribution of excipients plays crucial role in the propensity of a protein to self-associate

Applications of Arestat-T™

Formulation of labile proteins

developing new stable preparations of the next generation of therapeutic

High protein concentrations

proteins high therapeutic dose with reduced tendency to aggregate

Eliminating the need for a cold chain

ambient temperature stable formulations

Terminal sterilisation of solid doses

protein formulations able to withstand γ -radiation

Improving end user convenience

avoiding reconstitution of lyophilised dose forms

New routes of delivery

heat stable formulations for delivery via pens and pumps

Improved production economics

stabilization in-process; elimination of lyophilisation ; terminal sterilisation

Summary

- Stability is key to a successful protein application
- Formulation is key to protein stability
- Stability can be improved by optimisation of key formulation parameters
- Arecor provides additional bespoke solutions to achieve superior protein stability
- Arecor works in close partnership with Biotech and Pharma companies