

Monitoring the Transition from dimeric to hexameric Insulin

For demonstrating the monitoring of oligomerization via DLS, insulin was used as a classic example. Transition from a dimeric to hexameric state in presence of Zn²⁺is well known.



Monomer 1

absence of Zn²⁺.

Monomer 2



The expected outcome, hexamerization, but with unknown kinetics at the used conditions.

An Experiment for dimeric to hexameric transition of insulin in presence of **zinc ions** was prepared by loading of 12 aliquots on a Douglas Instruments vapor diffusion plate. Hexamer formation starts immediately in presence of zinc and were running for 72h at a constant temperature of 25° C. Insulin, 3.5 mg/ml in a **Zn²⁺** containing buffer







After plate loading the dimer-hexamer transition of insulin was monitored via in plate DLS.

DLS were measured in three measurement series (scans). First data were recorded immediately after plate loading (t_0) the second series after 11h delay time (t_0+11h) and the last series 72h after loading $(t_0 + 72h)$. The size changes is indicated also in the mean autocorrelation function (ACF). The overall shape of the ACF also indicates the uniformity of the particles in solution.

Mean autocorrelation function comparison of dimeric and hexameric insulin



The first and last scans are plotted together. Size differences of dimers and hexamers are clearly visible, PD values indicate that the dimer/hexamer transition was quantitative. DLS measurements taken at an intermediate state $(t_0 + 11h)$ where ~50% of the dimers also show size changes. However, the existing mixture of particles of different sizes (possibly dimers, tetramers and hexamers) is measured as a single peak of average particle size (page 4). The mixture is only indicated by an increase in the polydispersity index compared to the PD at t_o.

to hexameric state /Insulin lispro 14 hexameric insulin dimeric insulin ΰ Temperature (deg. (uuu) <u>2.37 nm</u> 1.92 nm +/-Sadius (+/- 0.02 nm 0.02 nm t₀ + 72h t₀ 4 0.0 2.5 10.0 Msmt. no 12.5 15.0 17.5 5.0 7.5

Size difference of dimeric

3



The transition is comparably slow, DLS measurements taken at t_0 showed no change in size. It was not expected that the transition would be so slow, so the monitoring time was extended.

The slow but continuous transition from dimers to hexamers eventually leads to intermediate mixtures with different ratios of monomer and oligomer concentrations.

The measured particle size at $t_0 + 11h$ of the dimer and hexamer mixture lies between dimers (1.9 nm) and hexamers (2.37 nm) at 2.02 nm. It is unlikely that a homogeneous tetrameric state was present in solution but a dimer/tetramer/hexamer mixture at different concentration ratio, indicated by the PD at $t_0 + 11h$ in comparison to t_0 .

Each detected particle size has an additional value the full width at half maximum or the polydispersity index (PD). It indicates the presence of such mixtures in the case of insulin dimers and hexamers.

The PD value is an indicator of mixtures of particles of different sizes in solution when the size differences are below the resolution limit of the DLS. This limit does not allow two particle sizes to be distinguished and shown as separate peaks because the fastest larger particles are as fast as the slowest small particles. The average diffusion velocity is then mixed and the sizes cannot be separated. On the other hand, the peak is broadened. This is indicated by the PD values and can be used to identify the presence of particle size differences.



Mixture of dimeric and hexameric state (t_0+11h)



Uniform hexameric state (t₀+72h)







Photon Count Rate for Transition Monitoring

The dimer/hexamer transition is a comparably slow process. Although the photon count rate is the most sensitive indicator for changes in a sample, rate are visible when DLS measurements are taken at time intervals of several hours.

Uniform dimeric insulin (t_0) av. photon count rate ~105 kHz



Mixture of dimeric and hexameric state (t_0+11h) av. photon count rate ~111 kHz



Uniform hexameric state (t_0 +72h) av. photon count rate ~145 kHz



Photon Count Rate versus Measurement Number



The differences in the photon count rate but also the remarkable stability at short observation intervals (~1h to 5h). That a transition happens at all becomes clear only when measurements were compared which were recorded over a significant longer time.