Evaluation of Assembly and Disassembly Behaviour of Drug-Loaded Peptide Nanoparticles

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Introduction

Elastin-Like Polypeptides (ELPs) are repetitive artificial polypeptides. The most common of ELP is in the form $(VPGXG)_n$ where X is a guest residue, and n is the number of pentapeptide repeats. ELPs in the form of $(VPGXG)_n$ exhibit an inverse temperature phase transition (T_t), meaning that below this temperature they are soluble in aqueous solution, and above the T_t they undergo an aqueous demixing phase transition resulting in aggregation of the ELP. The tunable properties and the T_t of the ELP make them well-suited for drug delivery within the body. Figure 1 shows the reversible phase transition behavior of ELPs.

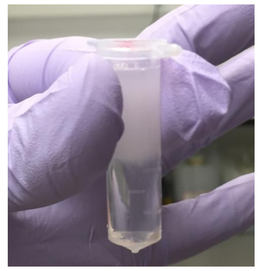


Figure 1: An image of the reversible phase transition behavior of ELPs

Methods and Materials

- ELPs aliquots are kept frozen at -80C and once thawed they need to stay in ice to make sure that they do not undergo preliminary T_t, to ensure the most accurate results.
- Both ELP stock solution and the drug solution, in this case Sildenafil (a well known vasodilator), are individually combined with 1xPBS buffer to determine the baseline assembly and disassembly behaviour of both the ELP and the sildenafil individually.
- ELP is then combined with the sildenafil and then diluted into the 1xPBS to create a solution to test the assembly and disassembly of the drug-loaded nanoparticles.
- A solution is then placed into the dynamic light scattering machine (DLS), and the intensity of the light scattering is measured, which is then used to derive volume. Figure 2 shows the special DLS cuvette.
- The DLS machine (Figure 3) measures Brownian Motion, the constant movement of particles due to collisions with other particles and liquids, to determine the size and therefore intensity of the particles.

Figure 2: An image of the special cuvette carrying the mixture of ELP and Sildenafil

Figure 3: An image of the DLS instrument used in the lab.

DLS Instrument Theory

- Brownian motion is the movement of particles due to random collisions with the liquid that surrounds the particle, therefore the particles are always moving.
- An important part of Brownian motion for DLS is that small particles move quickly while large particles move slowly.
- Due to the movement of the particles, constructive and destructive phase addition of scattered light will lead to dark and bright area fluctuation (Figure 4), therefore it is said that the intensity at any particular point fluctuates.
- These fluctuations and the idea that large particles will lead to slow fluctuations and small particles will have quick fluctuations is what the DLS machine uses to calculate a size distribution.

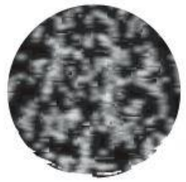
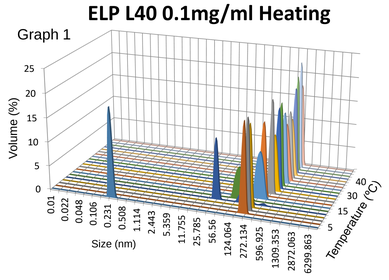
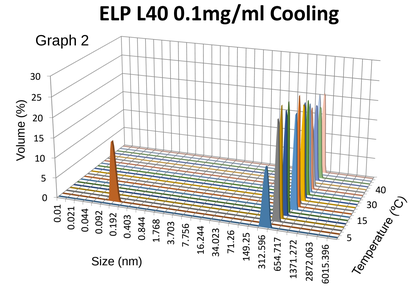
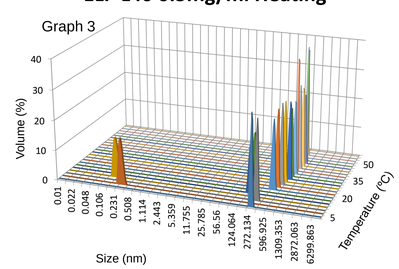


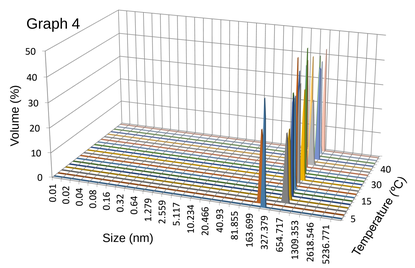
Figure 4: An image of scattered light exhibiting bright and dark area fluctuations as found in the Zetasizer Nano Series User Manual (Malvern Instruments LTD., 2003, 2004.)

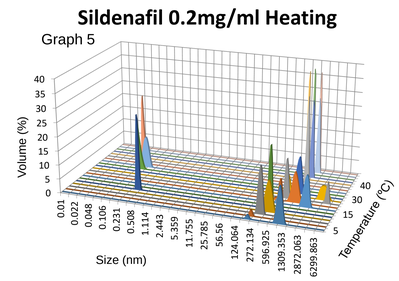
Results

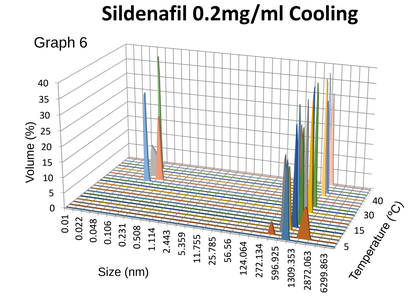




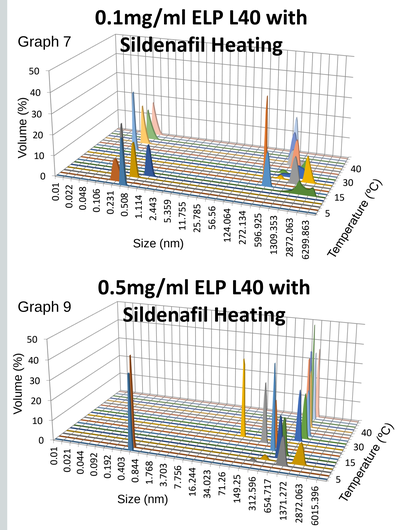


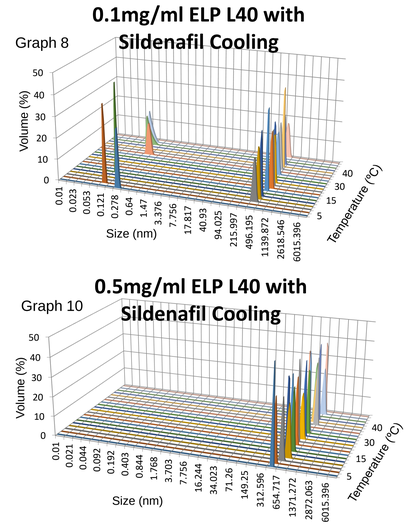






Graphs 1-6: These graphs are the baseline results of the heating and cooling behaviours of ELP L40 and Sildenafil.





Graphs 7-10: These graphs are the result of ELP combined with the Sildenafil at two different concentrations of ELP.

Conclusions

- The assembly behaviours of the 0.1mg/ml ELP L40 combined with the sildenafil solution varied from that of the assembly behaviours of just the ELP. The disassembly behaviours of the same ELP concentrations with sildenafil correlated with the ELP only solution, though the size of the ELP with the drug was larger and that might imply that the drug was loaded inside ELP particles.
- Similarly, the assembly behaviours of the 0.5mg/ml ELP L40 combined with the sildenafil solution related to the results of just the ELP though the size of the particles containing the drugs were much larger. The disassembly of the ELP with the sildenafil also correlated with that of the ELP only solution, though the particles were almost twice as large.
- The next step in this research is to determine quantitatively how much drug is loaded into ELP nanoparticles, and then evaluate the drug release profile from ELP nanoparticles/

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