Formulation of Four Peptide Solutions in 15 Days

Background

Custom designed oligopeptides are an increasingly accessible option in peptide-based technologies for pharmaceuticals. Following their synthesis, peptides are often subjected to HPLC purification and synthetic modification, which can require the use of non-aqueous media or salts requiring later removal. During the production of pharmaceutical peptides, spoilage can occur in the forms of aggregates, associates, and other means. Using ARGEN, we demonstrate the rapid formulation of four peptide solutions that are based on excipient effects correlated to increasing molecular weight. The first peptide was formulated in five days for optimal stability. The remaining three peptides were formulated in parallel over 10 days.

Peptide	# of A.A.'s	Mass (Daltons)	Hydrophilicity	[Peptide] (mg/mL)	pH	[MeCN] (%)	[DMSO (%)	[GnCl] (M)
A-1	7	850	Hydrophilic	2.5	6	25	15.0	2.0
A-2	7	850	Hydrophilic	1.0	6	25	20.0	3.0
B-1	36	4200	Hydrophobic	10.0	4	50	15.0	0.0
B-2	15	1700	Hydrophobic	--	8	63.0	10.0	0.0

Table 1: Peptide characteristics and optimal conditions determined by rapid formulation with ARGEN. Exact amino acid sequences are not reported due to disclosure conditions with the supplier, but peptides are grouped by their reported hydrophilicity. Based on peptide primary structure, A-group peptides were predicted to be hydrophilic, and B-group peptides were predicted to be hydrophobic. Their excipient-dependent behavior was consistent with this designation. (%) is percent by volume. Peptide B-2 was not soluble enough to find an optimal peptide concentration for long-term storage. Peptide B-2's poor solubility precluded it from a concentration study.

Methodology

Monitoring Peptide Aggregation with ARGEN:

Four peptides of unknown primary structure were examined against a panel of solution additives. Excipients included dimethyl sulfoxide (DMSO), acetonitrile (MeCN), and guanidinium chloride (GnCl). Other conditions investigated were pH and peptide concentration.

Each peptide was dissolved to 2 mg/mL in a 1:1 H_2O:MeCN cosolvent solution which was adjusted to pH 7 before peptide dissolution, as prescribed by the peptide's provider. This solution was used to prepare 1 mg/mL solutions of the peptide with an excipient. These conditions included guanidine hydrochloride from 0-4.3 M, DMSO from 0-20% by volume, and MeCN from 25-75% by volume. All solutions were held at 30 °C for up to 48 hours under constant monitoring by ARGEN.

Once the afore described assays were analyzed, a stock solution with each additive at its optimal concentration was prepared for each peptide. Each peptide was dissolved in its respective buffer at 1 mg/mL, and pH was adjusted with dilute NaOH and HCl solutions to identify the optimal pH

between 2 and 10. This was determined by characterizing the degree of aggregation for each peptide at each pH level over a 48-hour period of aggregation monitoring at 30 °C. The formulated sample with the least aggregation after 48 hours of monitoring correlates to the optimal storage condition (variables included varied pH and varied concentrations of additives). Solubility testing determined that all peptides struggled to dissolve at concentrations greater than 10 mg/mL.

Results

Guanidinium Chloride:

Guanidinium chloride was shown to have stabilizing effects at intermediary concentrations for the "A" group peptides. Stability maxima were observed between 2-3 M of salt (Figure 1). The light scattering signature of aggregation is steady and relatively linear. This denotes a steady increase of Normalized molecular weight from its monomeric, unaggregated state, to that of a population of dimerized aggregates. Kinetic analysis showed that peptides A-1 and A-2 increased in stability by 250 and 350 times, respectively, with the addition of the optimal concentration of guanidinium chloride.

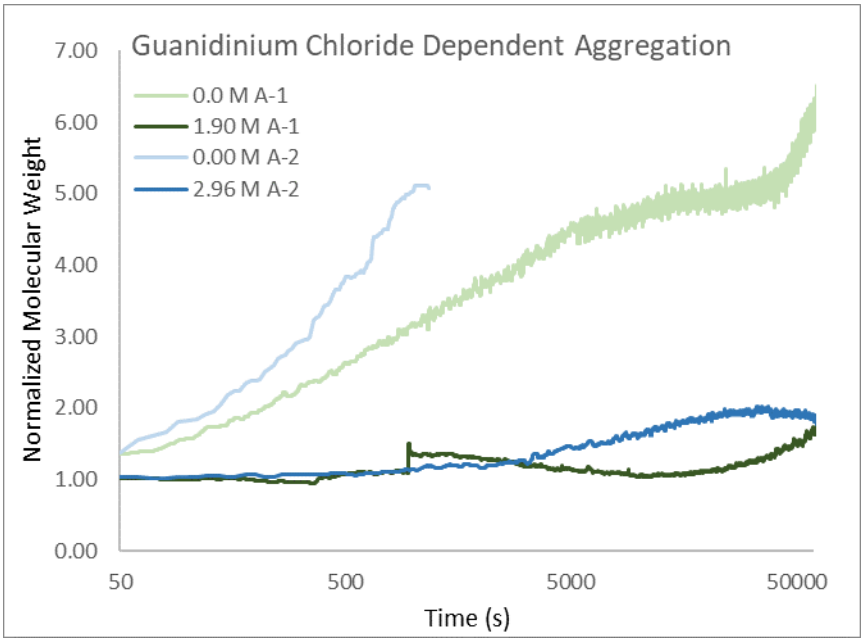

Figure 1: Hydrophilic peptides exhibited increased stability as ionic strength of guanidinium chloride increases. Their optimal stability was achieved between 2 and 3 M

Both "B" group peptides, B-1 and B-2, precipitated almost immediately following the addition of saline solution. With the understanding that the B group peptides are hydrophobic, this is not unexpected. Though guanidinium is a chaotrope, its nature as a salt increases the solution's polarity, destabilizing the sparingly dissolved hydrophobic peptides. Further investigation would greatly reduce the concentration of GnCl for these peptides to analyze stability in a micromolar regime.

***Dimethyl Sulfoxide & Acetonitrile**:*

Across all four peptides studied, increasing concentration of DMSO resulted in increased peptide stability. Three peptides showed stability maxima, between 0 and 20% DMSO by volume. Peptide A-2 showed maximal stability at 20%, implying if this set of assays were to be extended to a greater proportion of DMSO in solution, this peptide's stability could further increase. Regardless of hydrophilicity, the addition of DMSO was beneficial for stability.

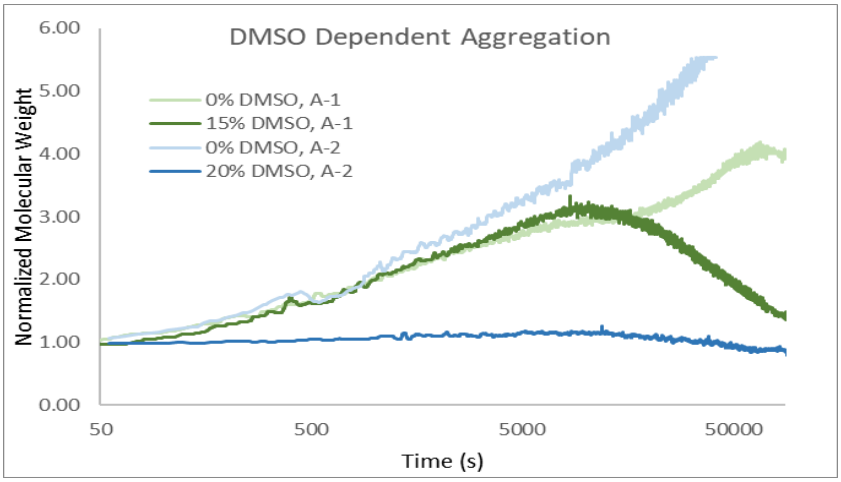

Figure 2: Peptide A-2 greatly increased stability with increased DMSO concentration. Peptide A-1 showed similar behavior in both conditions, until approximately two hours into the experiment where the DMSO containing solution dissociated back to monomers.

The stability gains achieved by the addition of DMSO were not observed in the case of the MeCN addition. MeCN caused rapid aggregation of the A group peptides, but it caused stabilization of the B group peptides. This can be accounted for by changes in solution polarity. Decreased solution polarity can decrease enthalpic penalties of solvation for hydrophobic solutes.

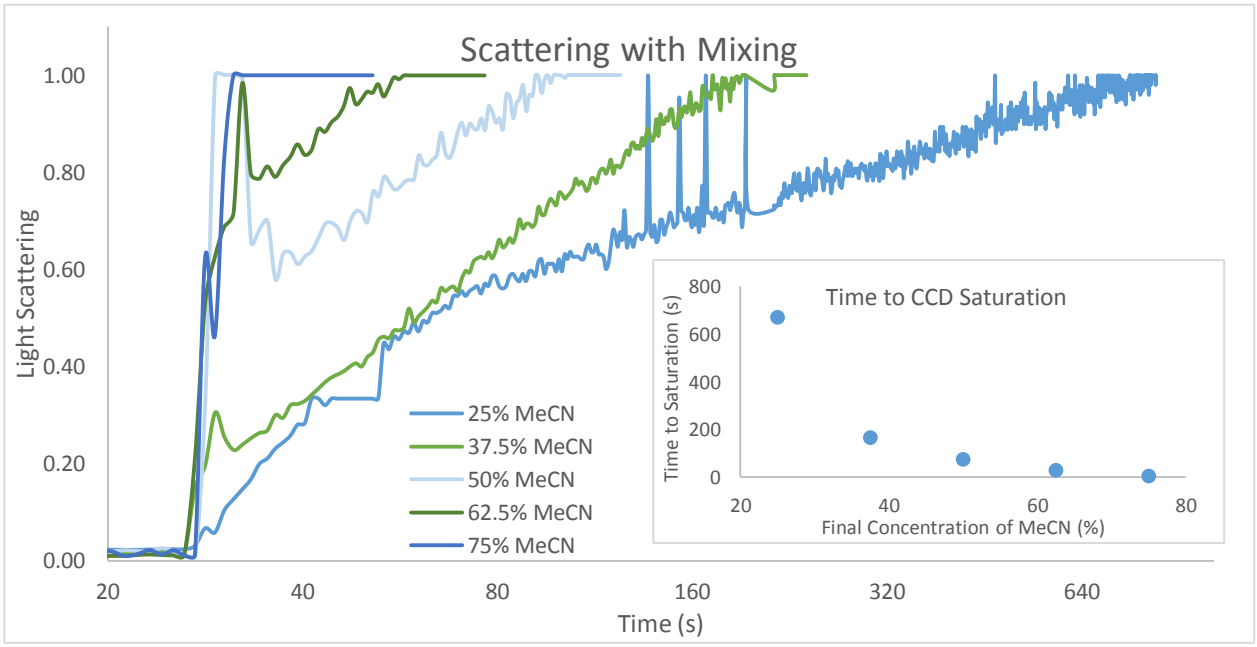

Figure 3: (peptide: A-1) As the final concentration of acetonitrile increases, the CCD reaches saturation faster. There are two explanations for this. 1) A-1 is quickly aggregating due to a change in the solution polarity, or 2) the addition of the MeCN:H2O cosolvent solution drastically changes the optical properties of the solution when the peptide is present.

***pH**:*

pH dependent stability was highly variable between samples. More extreme pHs tended to represent increased stability against aggregation, or conversion to an insoluble species. Extreme pHs could also be problematic because of acid or base catalyzed degradation pathways. In most cases, there were moderate pHs in which samples demonstrated relatively stable behavior and were not prone to hydrolysis.

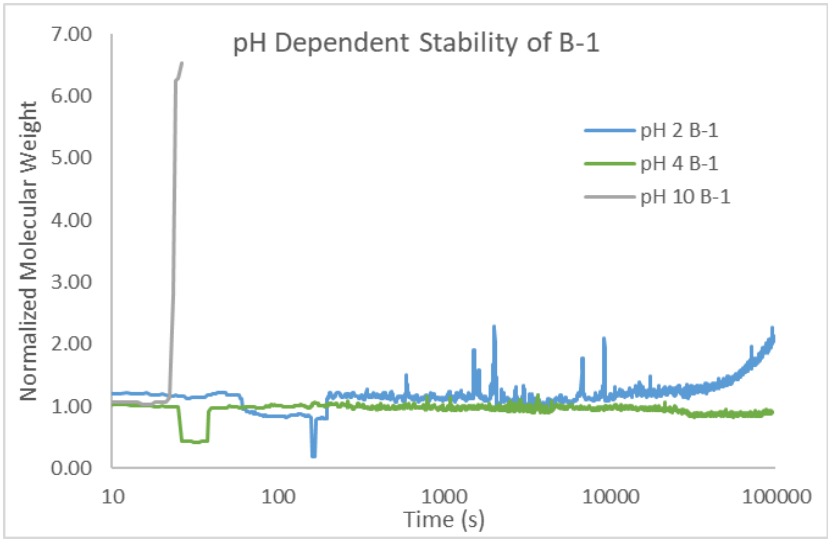

Figure 4: B-1 rapidly precipitated in basic conditions. At pH 2, gradual aggregation occurs at the end of the experiment, but there was almost no aggregation observed at pH 4. (Data Not Shown) pH 6 and pH 8 showed identical behavior as pH 10 but at a slower rate. Both are consistent with observation of pH dependent precipitation from change in protonation state rather than irreversible aggregation.

***Peptide Concentration**:*

Group A peptides showed decreasing stability with increasing concentration of peptide. Since these peptides easily dissolve into their solution, they can effortlessly explore conformations with favorable intermolecular interactions. Different from Group A peptides, Peptide B-1 shows colloidal stability. As the concentration of peptide increases, the solution is more stable. This is normally sustained by intermolecular repulsion preventing peptides from associating.

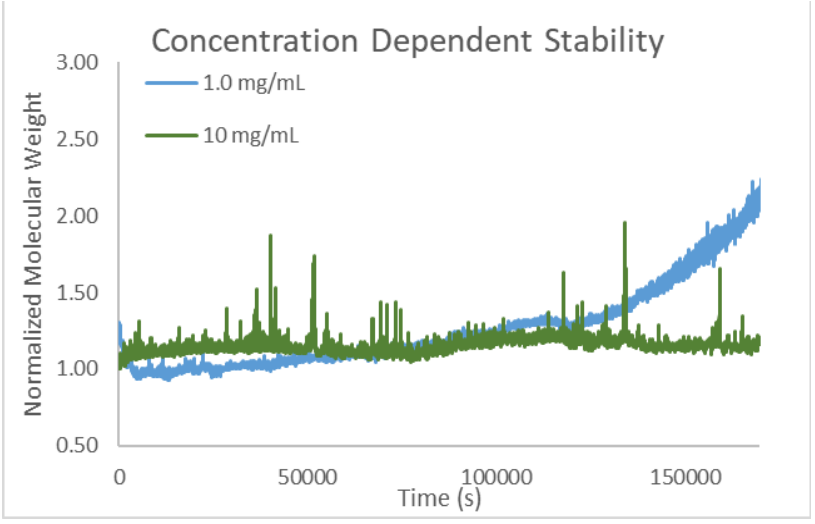

Figure 5: Peptide B-1 exhibited colloidal stability. The stability granted by higher concentration is only a slight increase and is best observed by the gradual aggregation at the end of the experiment when the dilute sample dimerizes.

Conclusion:

- ARGEN was used to formulate four pharmaceutical peptides for long-term storage in aqueous form based on continuously collected data and a kinetic approach to analysis and data interpretation
- Future research could utilize all 16 of ARGEN's cells to better finely tune the formulation and probe for interactions between excipients

- Using ARGEN's Kinetic data, it was also possible to quickly rule out certain excipients such as guanidinium chloride for the hydrophobic B-group peptides and acetonitrile for the hydrophilic A-group peptides
- Lastly, future research could look into the effects of more exotic additives that would be beneficial given a known primary structure

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