F19CHa Amyloid Beta Characterization Report

Abstract:
Samples of lyophilized F19CHa, amyloid beta peptide, were received for biophysical characterization at the Canadian Center for Hydrodynamics. Previous studies of F19CHa using NMR studies have showed that dodecamers form in water while silver-stained SDS-PAGE and crystallography report that F19CHa assembles into hexamers. The objective of this experiment was to determine distinct amyloid beta self-association behaviours using analytical ultracentrifugation (AUC).

Methods:
1, 3, and 9 mM samples of F19CHa were prepared in Milli-Q water at room temperature (23 ºC). The measurements are listed in **Table 1**.

Table 1*: Measurements used for F19CHa 1, 3, and 9 mM concentrations*

F19CHa Concentration	Provided Sample	MiliQ Water
1 mM	1.07 mg	600.68 uL
3 mM	1.38 mg	258.24 uL
9 mM	3.30 mg	205.84 uL

UV-visible absorbance measurements were performed with a Genesys 10S bench-top spectrophotometer in a 60 µL cuvette to determine the optimal wavelength to measure F19CHa in the AUC (**Figure 1**). The wavelengths selected for AUC measurements of each concentration can be found in **Table 2**. All experiments were performed using a Beckman-Coulter Optima AUC instrument at the Canadian Center for Hydrodynamics. The samples were loaded into 3 mm titanium centerpieces (Nanolytics, Potsdam, Germany).

Table 2*: Absorbance measurements taken with Genesys 10S benchtop spectrophotometer and AUC*

F19CHa Concentration	Absorbance (1 cm)	AUC Absorb. (25 krpm, 1 cm)	Difference	AUC Absorb. (60 krpm, 1 cm)	Wavelength
1 mM	1.253 OD	1.14 OD	0.11 OD	1.14 OD	237 nm
3 mM	1.230 OD	1.01 OD	0.22 OD	1.00 OD	244 nm
9 mM	1.291 OD	0.79 OD	0.50 OD	0.79 OD	268 nm

An initial experiment was run to determine the optimal rotor speed. The F19CHa samples were run at 25 krpm, which showed no sedimentation. The cells were removed from the AUC, shaken to redistribute the protein, and placed back into the AUC. The speed was then increased to the maximum of 60 krpm and the experiment was run for 16.7 h at 20 °C. All samples were analyzed by sedimentation velocity analytical ultracentrifugation (SV AUC) employing UV/visible optics, measuring in intensity mode at the wavelengths indicated in Table 2. All acquired data were analyzed

with UltraScan-III [1]. To enhance accuracy, a two-dimensional spectrum analysis (2DSA [2]) was implemented to eliminate noise invariant with respect to time and radius, as well as to precisely fit the meniscus position. A final 2DSA refinement, encompassing ten iterations, was performed for each dataset as well as a Monte Carlo [3] refinement. In the initial analysis, the 2DSA simulated fits were not aligning with the raw data, giving poor RMSDs. By looking at the data in time increments, it was apparent that the association of the amyloid beta molecules was changing throughout the duration of the experiment. In order to see the time dependent self-association, the raw pseudo-absorbance data was split into three separate edit profiles: early, middle, and end (**Figure 2**). The parameters used for the analysis, as well as the scan range for each edit profiles is listed in **Table 3**. The same analysis methods mentioned previously were then repeated.

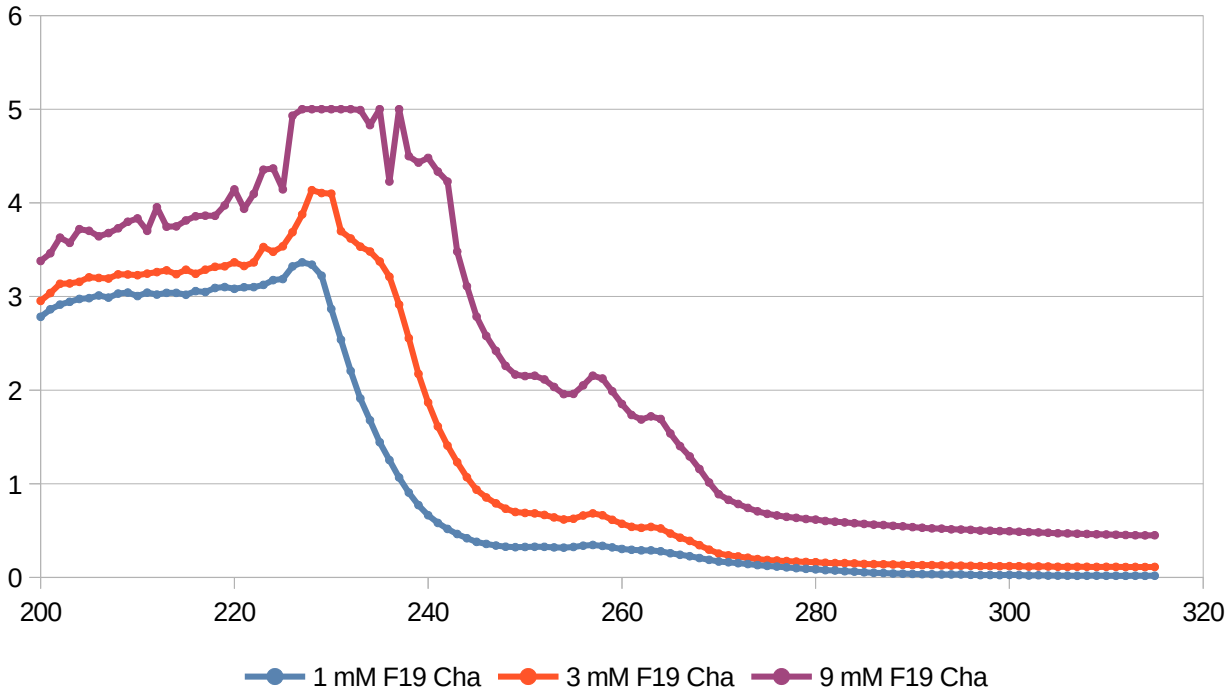

***Figure 1**: UV-visible absorbance measurements with Genesys 10S bench-top spectrophotometer*

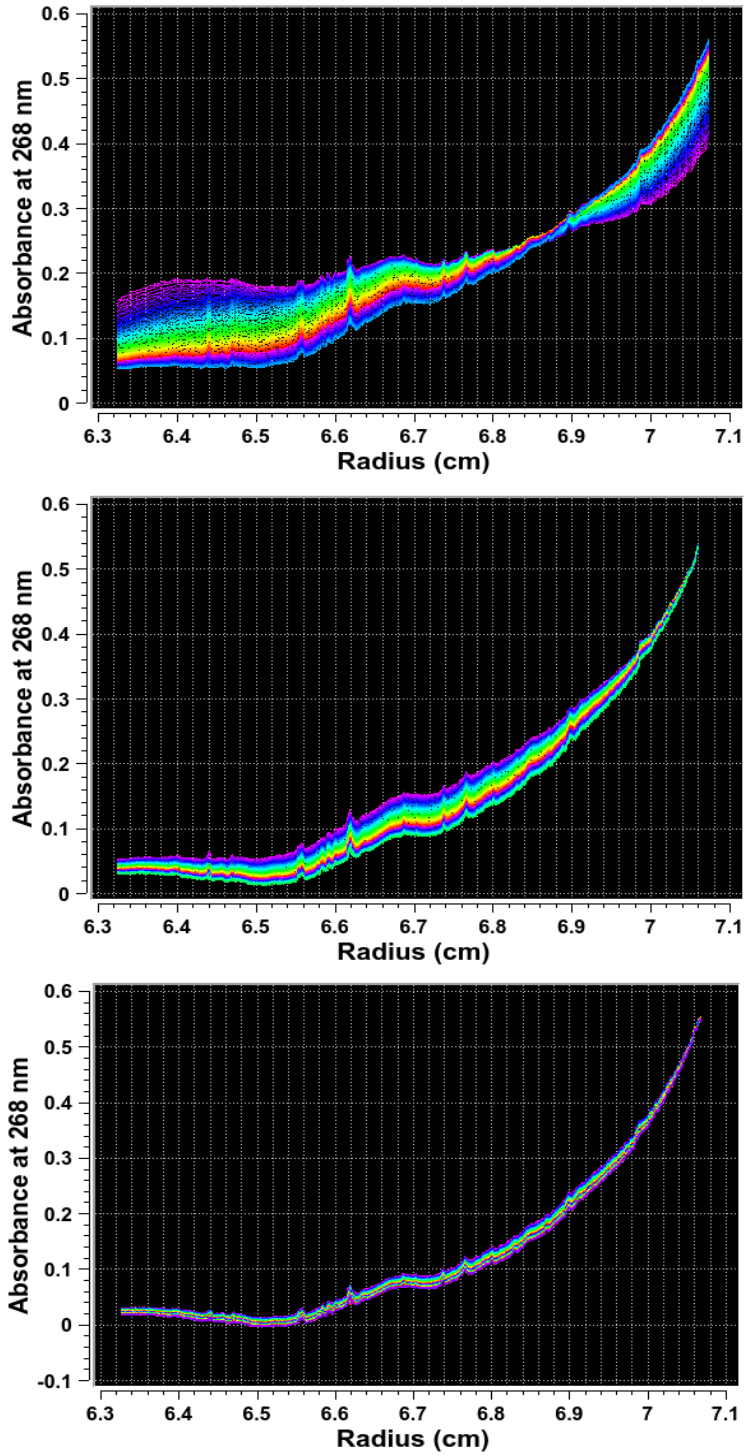

***Figure 2**: Pseudo-absorbance early, middle, and end (top to bottom) scans for 9 mM F19CHa run at 60000 rpm.*

***Table 3**: Parameters for SV AUC*

Sample	Edit Profile	Start Scan	End Scan	S20 min	S20 max	Resolution	f/f0 min	f/f0 max	Resolution
1 mM F19CHa	Early	5	90	0.1	1	100	1	3	100
	Middle	95	185	0.1	1	100	1	3	100
	End	185	end	0.1	1	100	1	4	100
3 mM F19CHa	Early	5	90	0.1	1	100	1	3	100
	Middle	95	185	0.1	1	100	1	4	100
	End	185	end	0.1	1	100	1	4	100
9 mM F19CHa	Early	5	70	0.1	1	100	1	3	100
	Middle	75	155	0.1	1.5	100	1	6	100
	End	155	end	0.1	2	100	1	4	100

Results:

The UV absorbance spectra of 1 mM, 3 mM, and 9 mM F19CHa shows Mie scattering, indicating aggregation of the peptide directly after the sample preparation (Figure 1). This effect is seen to increase with an increase in concentration. Further aggregation of F19CHa was confirmed by the AUC UV absorbance measurements. In Table 2 a decrease in absorbance is observed in the AUC compared to the spectrophotometer reading, indicating a loss of material proportional to the concentration of the sample. There was, however, virtually no change in absorbance between the experiments run at 25 krpm and 60 krpm which suggests that the aggregation remained consistent in between runs. The analysis of F19CHa using Ultrascan III showed high RMSD values for the 2DSA simulated fits for the raw data. By separating the scans into early, middle, and late edit profiles (Figure 2) the data was able to be successfully analyzed with low RMSD's (< 0.004). The fact that the separation of scans according to time had a significant improvement on the fitted data suggests chemical non-equilibrium conditions of the peptide, consistent with time-dependent aggregation. In each concentration the sedimentation species changed composition from early to end scan selections (Figure 3). Only the lowest concentration showed a general shift from a lower to higher sedimentation coefficient as a function of time. The higher concentration samples showed different, but inconclusive patterns, which also incurred higher aggregation losses. When comparing integral distribution plots from early scans, the sedimentation profile increased to larger sedimentation coefficients as the concentration was increased (Figure 4). In the 1 mM F19CHa sample, an increase in the frictional ratio was also observed from the early to end edit profiles (Figure 5). This confirms the oligomerization in a fibrillar conformation.

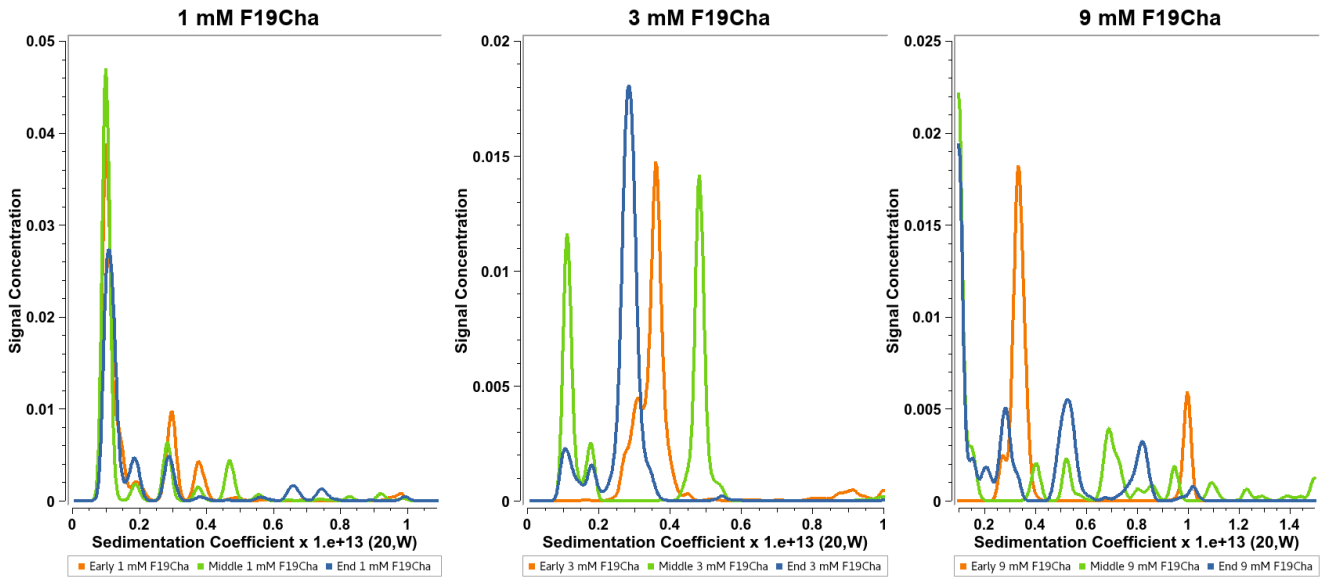

Figure 3: Sedimentation distribution of 1 mM, 3 mM, and 9 mM (left to right) early, middle, and end scans.

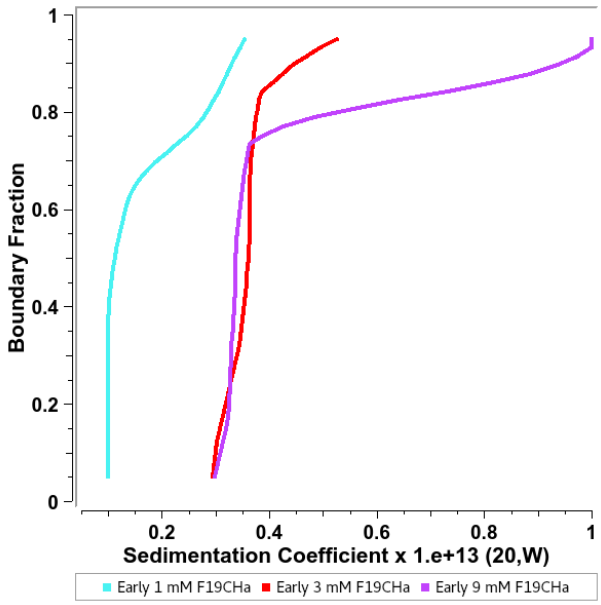

Figure 4: Sedimentation integral plot of early edit profiles for each concentration

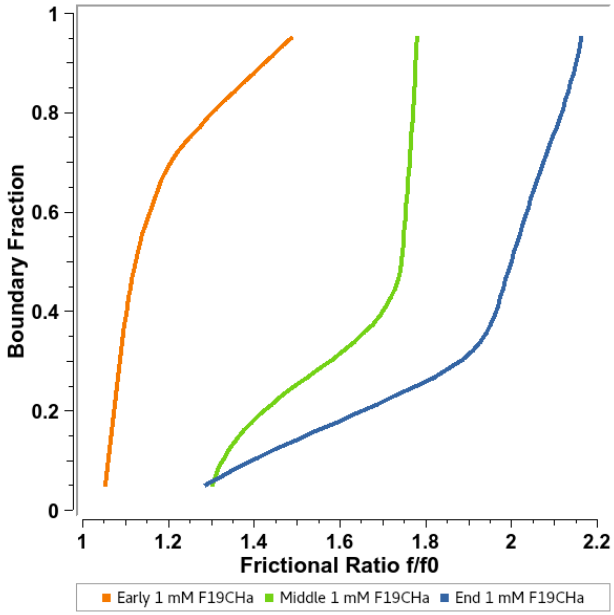

Figure 5: Frictional ratio integral plot for 1 mM F19CHa early, middle, and end edit profiles

Discussion:

The F19CHa peptide, due to its low monomeric molar mass, is at the limit of detectability at 60 krpm. This is aggravated by the relatively high partial specific volume, which prevents a useful sedimentation signal. Simulations of the sedimentation coefficient for the peptide, based on molar mass, suggests an s-value of 0.28 for the monomer, 0.93 for the hexamer and 1.47 for the dodecamer. Due to these low s-values, the resolution of these species is rather limited, and only approximate. In addition, the time dependent nature of the aggregation complicates a rigorous s-value analysis. The clearest indicator for aggregation is the Mie scattering observed in Figure 1, and that spinning up to 25 krpm already precipitated the aggregates before the first scan was taken, reducing the absorbance. Nevertheless, we can detect sedimentation signal in the region of 0.1-1.5 s, consistent with the presence of monomer-dodecamer, with a prevalence of the monomer in all cases. This suggests that only the monomer-dodecamer oligomers are soluble, while the larger aggregates readily precipitate from solution even at low speed. A higher peptide concentration simply results in more aggregation, as documented by the clear trend in the absorbance difference values from Table 2. The heterogeneous composition of all samples measured at multiple concentrations may also explain the difficulties encountered in the NMR experiments.

1 Demeler B., and G. Gorbet. Analytical Ultracentrifugation Data Analysis with UltraScan-III. Ch. 8, In Analytical Ultracentrifugation: Instrumentation, Software, and Applications. Eds: Uchiyama S., Stafford W. F. and T. Laue. Springer, 119-143 (2016)
2 Brookes E, Cao W, Demeler B. A two-dimensional spectrum analysis for sedimentation velocity experiments of mixtures with heterogeneity in molecular weight and shape. Eur Biophys J. 2010 Feb;39(3):405-14. doi: 10.1007/s00249-009-0413-5. Epub 2009 Feb 27. PMID: 19247646.
3 Demeler B. and E. Brookes. Monte Carlo analysis of sedimentation experiments. Colloid Polym Sci (2008) 286(2) 129-137