Overcoming CD Spectroscopy Sample Preparation Challenges in Protein Structural Analysis for Biosimilars

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Supplemental findings presenting Far-UV CD spectra of 20 µM α-chymotrypsinogen A with a 40 mM SDS concentration achieved through a two-step process.

Figures S1–3 provide additional insights by illustrating discrepancies in spectra resulting from solutions mixed to attain final 40mM concentrations following a different mixing protocol, as depicted in Figure 1 within the main article text. Specifically, these figures showcase far UV CD spectra obtained from solutions prepared by combining 40 µM α-chymotrypsinogen A with a small amount of SDS solution, followed by mixing with an SDS solution in buffer to reach a final concentration of 40 mM SDS. Given that the protein and small amount of SDS were mixed in equal volumes, the resulting solution contained 20 µM α-chymotrypsinogen A and a 40 mM SDS concentration. Figures S1-3 exhibit the impact on far UV CD spectra for SDS concentrations ranging from 6 to 20 mM, maintaining a consistent initial protein concentration of 40 µM to ensure accurate measurements with minimal UV radiation exposure.

All spectra were acquired immediately after achieving complete mixing of the solutions. Notably, Figures S1–3 reveal substantial differences in the far UV CD spectrum of the protein solution containing a low concentration of SDS mixed with an SDS solution, in comparison to spectra obtained from the protein solution containing a 40 mM SDS concentration. Each obtained spectrum is individually compared with two reference spectra: the first recorded after mixing a 40 µM protein solution with a buffer, and the second recorded after mixing a 40 µM protein solution with an 80 mM SDS solution.

Figure S1 confirms that the spectra recorded after mixing a 40 µM chymotrypsin solution containing small amounts of SDS with an SDS solution, resulting in a final concentration of 40 mM, differ from the spectrum obtained after mixing a 40 µM protein solution with an 80 mM SDS solution, despite having the same final protein and surfactant concentration.

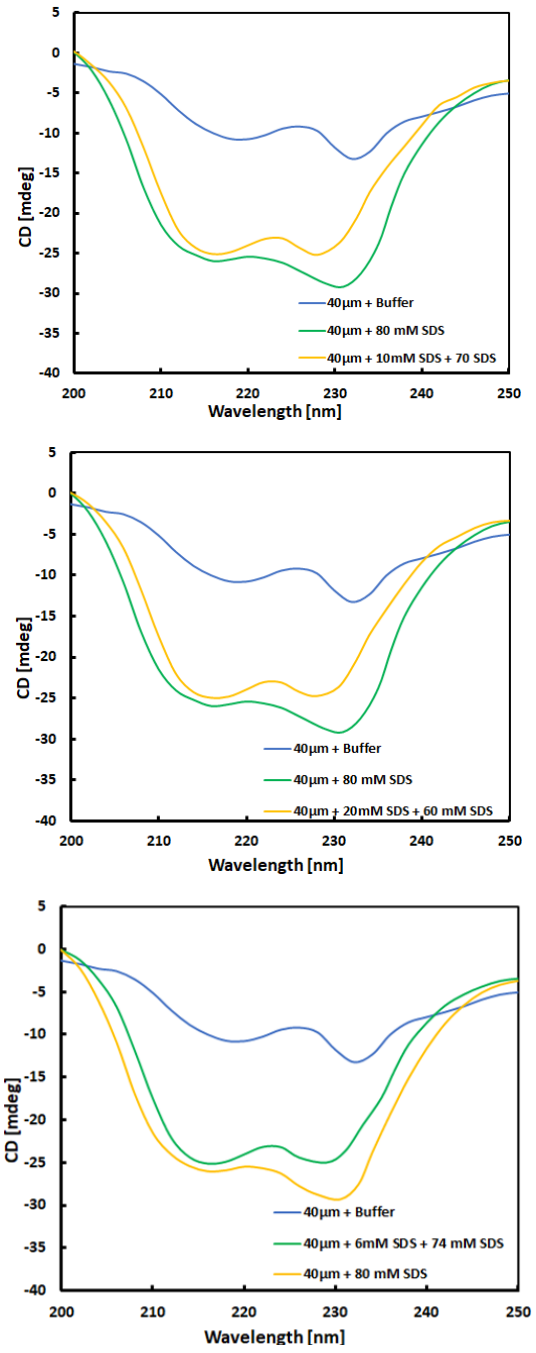

Figure S1 –3 Far UV CD spectra for all solution as shown detailed in legend of each plot.