

## **The correlation between gelatin macroscale differences and nanoparticle properties: providing insight to biopolymer variability**

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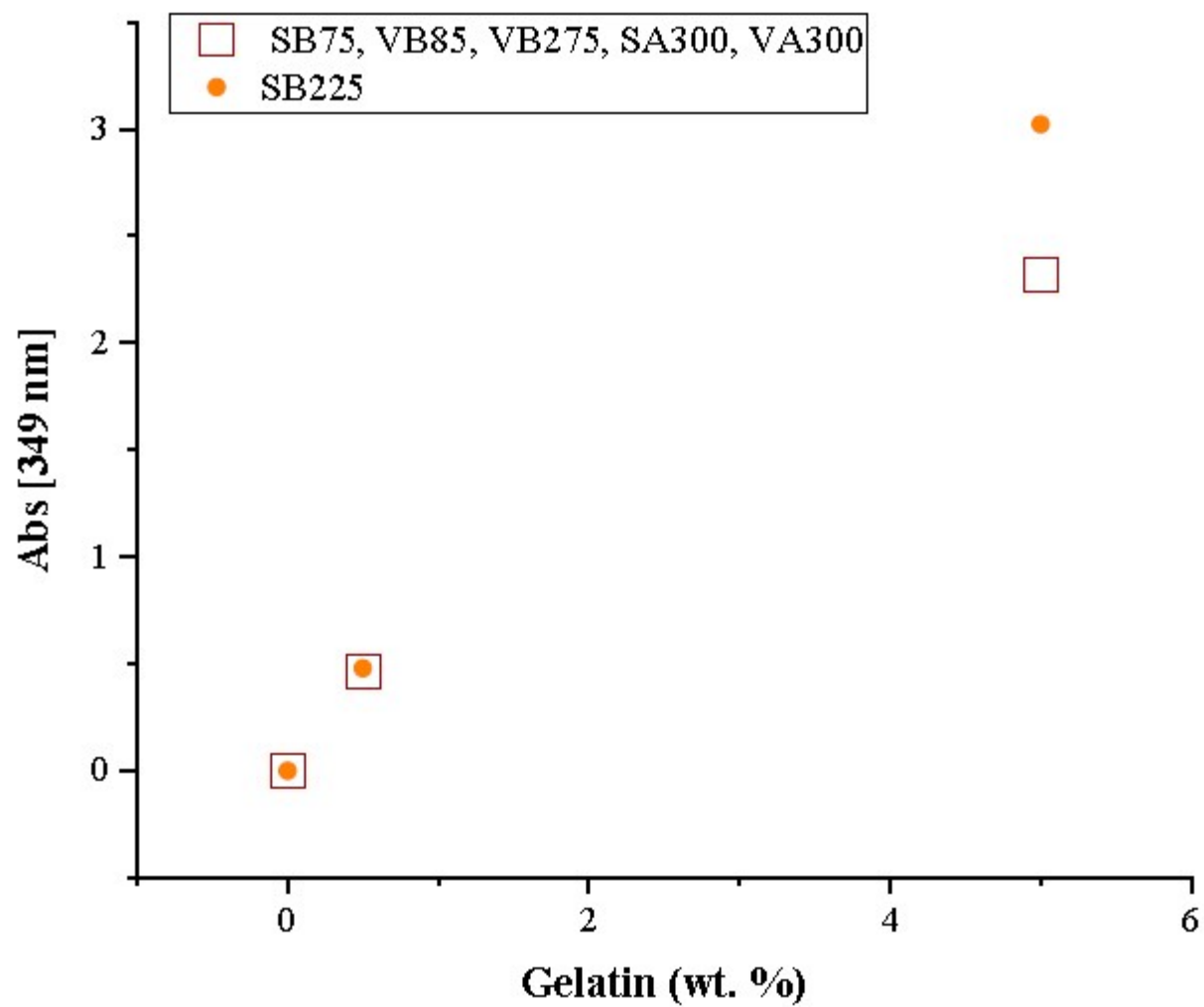
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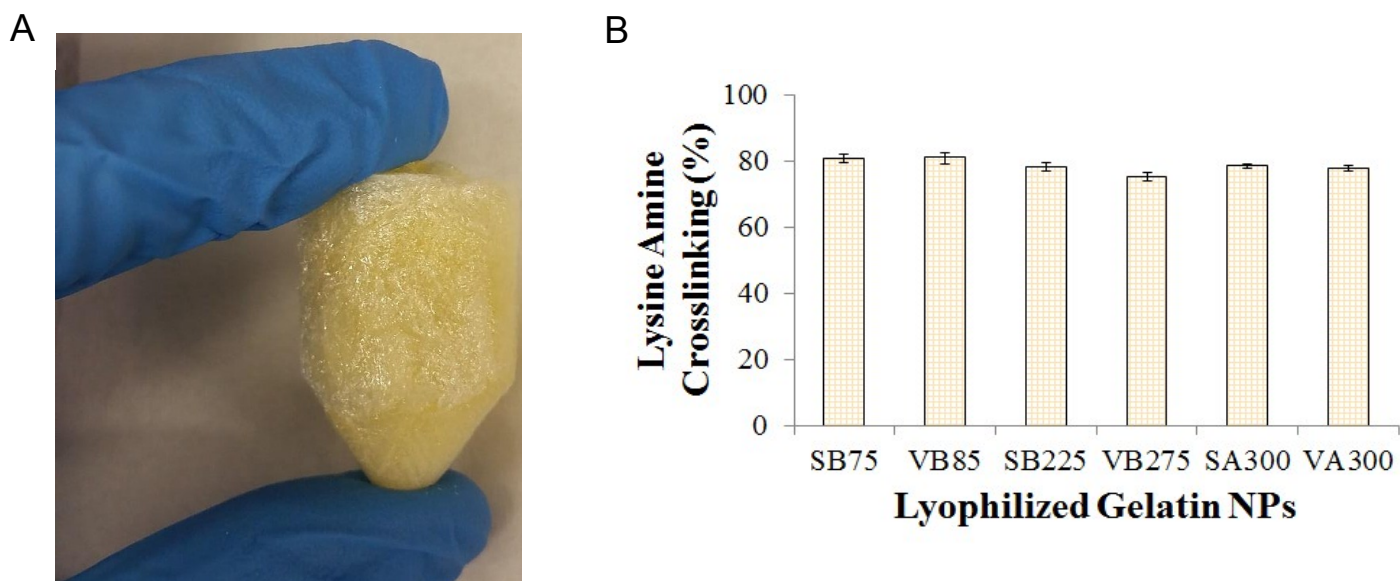
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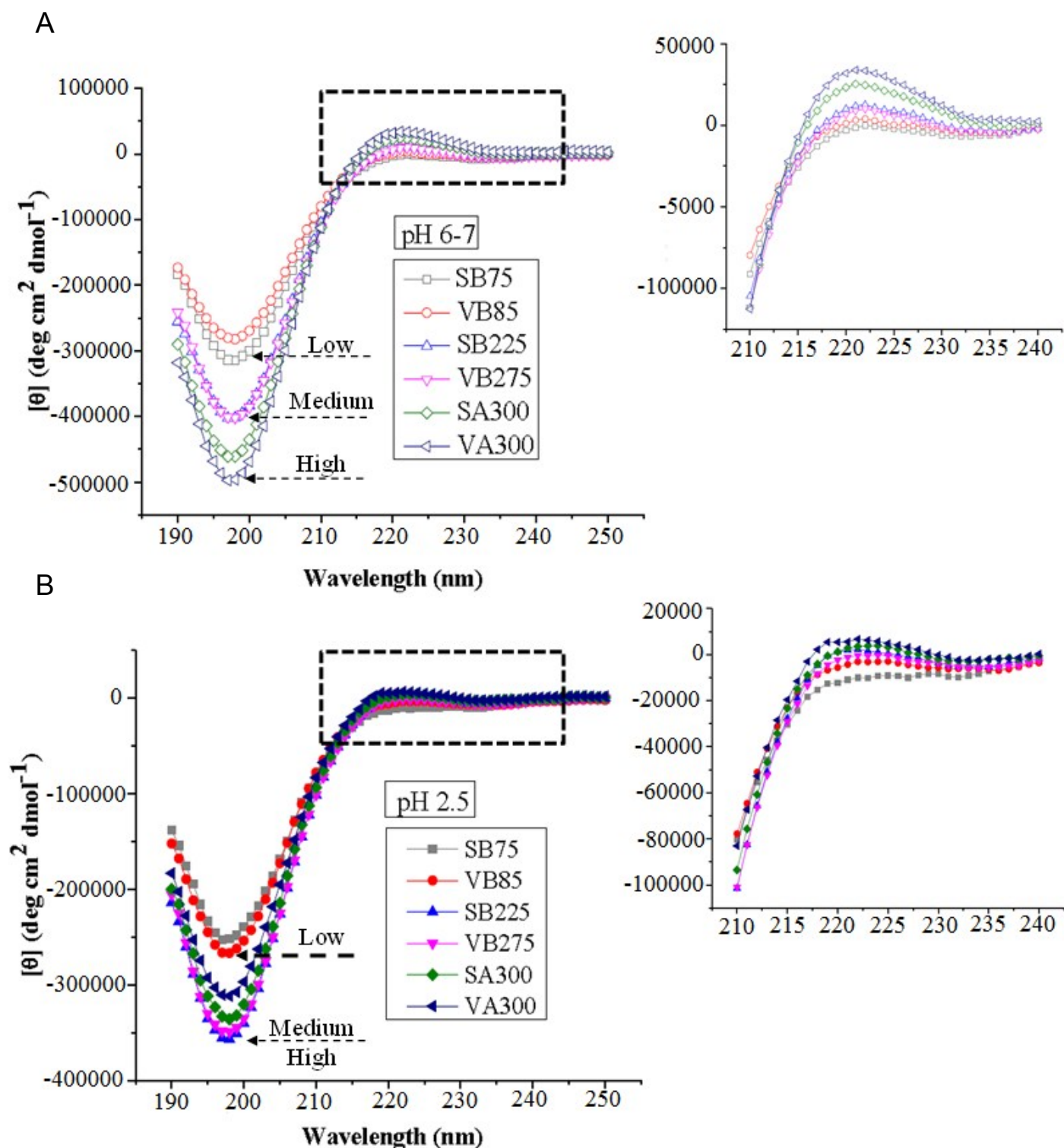
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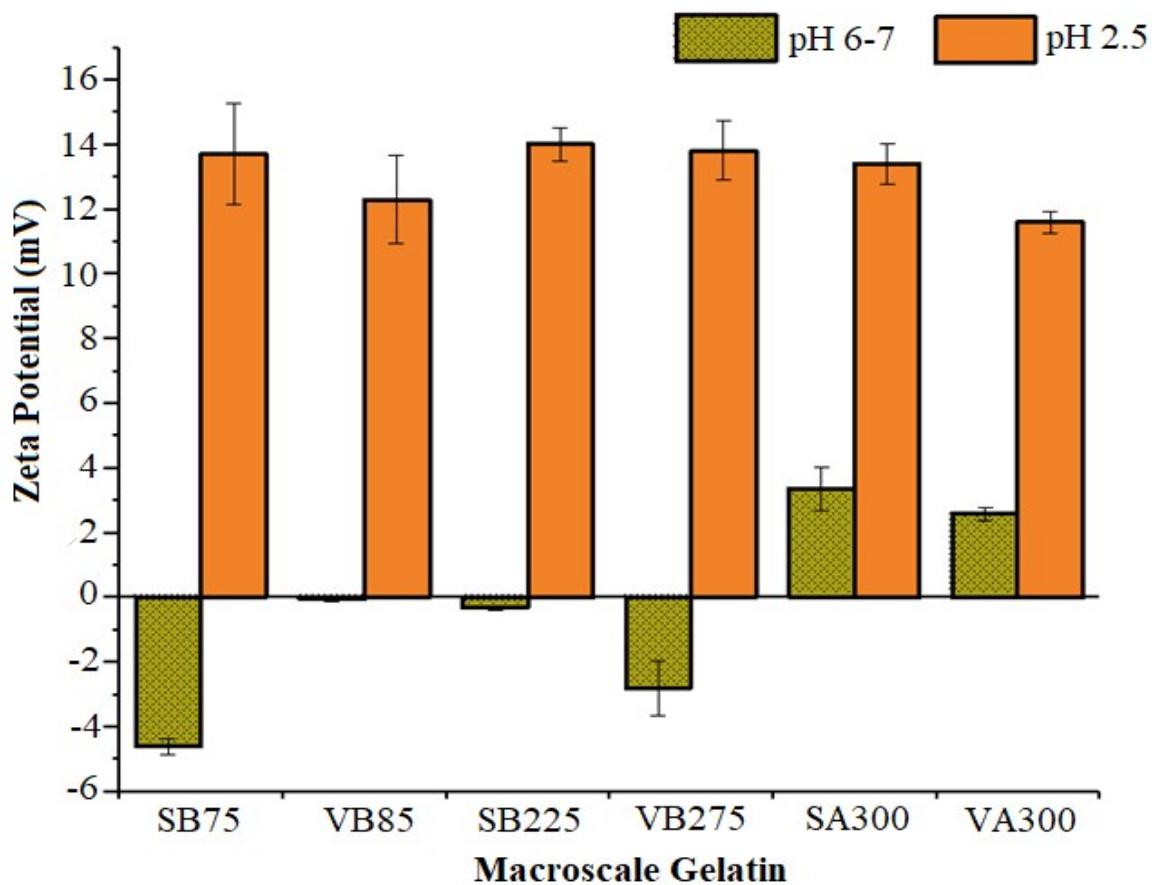
**Figure S1.** Increased gelatin concentration produces higher absorbance values using TNBS assay. SB225 at 5 wt. % shows a higher absorbance indicating more available lysine. n = 3 measurements averaged.



**Figure S2.** (A) Representative dry foam of gelatin NPs after lyophilizing solution for 2 days. (B) All lyophilized NPs were ~80% crosslinked.  $n = 3 - 5$  measurements per sample. Data represent mean  $\pm$  standard deviation.

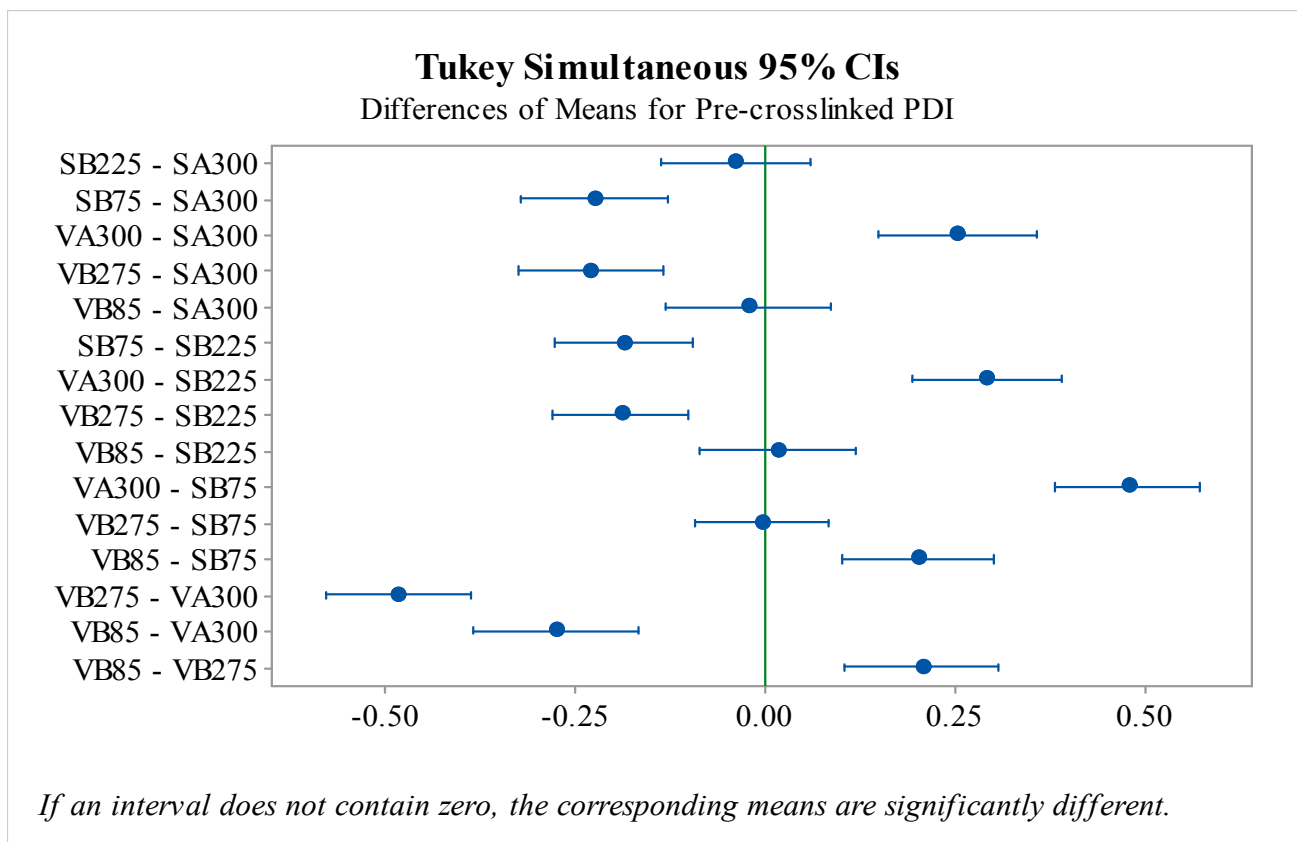


**Figure S3.** Circular dichroism confirms analogous macroscale gelatin structures in aqueous solutions. (A) Gelatins at pH 6-7 have three distinct regions of random coil intensity by Bloom strength. Insert reveals at most moderate triple helical content likely due to heat denaturation. (B) Gelatins at pH 2.5 have random coil (and triple helical) reductions indicating chemical denaturation and likely chain stacking due to protonation of amine groups. Insert reveal further reductions in triple helical content as well. All gelatin samples respond similarly in aqueous environments indicating analogous structures.  $n = 3$  separate trials with 3 scans per trial.



**Figure S4.** Macroscale gelatin zeta potential measurements reveal pH dependency. Type A and Type B gelatin have modest charge ( $0 \pm 4$  mV) in distilled water (pH 6-7). Acidic environments (pH 2.5) are significantly below gelatin pIs leading to protonation of amine groups and net positive charges.  $n = 3-5$  measurements per sample. Data represent mean  $\pm$  standard deviation.

explanation for random coil reductions in acidic pH. Gelatin's isoelectric point (pI) is the pH at which there is zero net charge. The pI of Type B gelatin is 4.7-5.4 while the pI of Type A gelatin is 7-9.<sup>1</sup> All gelatins have modest charges ( $\sim 0 \pm 4$  mV) (Figure S4) in distilled water (pH 6-7). In acidic solutions, amine groups undergo protonation resulting in net positive charges for all gelatins (Figure S4). Further, our results suggest protein charge and the degree of random coil are associated. One possible explanation is due to gelatin chain flexibility. Gelatin Type B chains can persist for a 2 nm distance before changing direction while Type A chains can persist for 10 nm before changing direction.<sup>2</sup> These highly flexible chains containing charged amine groups have a greater propensity to overlap and interact with negatively charged segments.<sup>3,2</sup> The intra chain association results in chain stacking at acidic conditions, which likely reduces some of the random coil intensity in the overall gelatin structure.



**Figure S5.** One-Way ANOVA with Tukey's pairwise comparison for gelatin pre-crosslinked PDI values. Statistical significance yields the same results for the pairs compared to Kruskal – Wallis test (Figure 5C). n = 2 separate trials with 3 to 6 measurements per trial included. Data represent mean  $\pm$  standard deviation.

$$^1R_{\text{minimum}} = 0.066 * M_n^{1/3} \quad (\text{Eq 1})$$

$R_{\text{minimum}}$  : minimum radius of a gelatin chain

$$D_H = 2 * R_{\text{minimum}} * x_{\text{gelatin chains}} \quad (\text{Eq 2})$$

$D_H$  : hydrodynamic diameter based on theoretical number of crosslinked gelatin chains

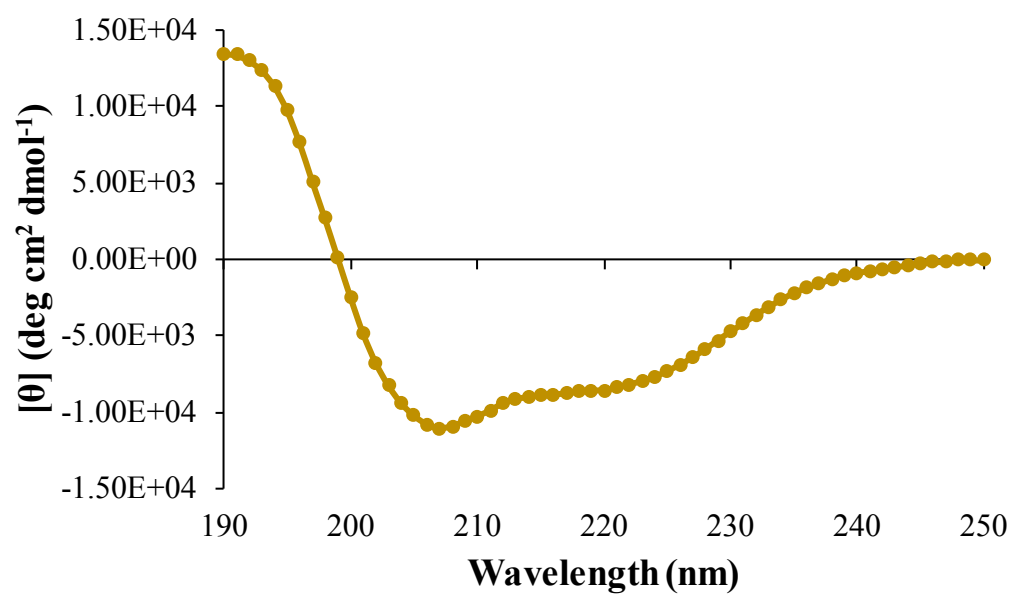
$x_{\text{gelatin chains}}$  : the number of gelatin chains that can be crosslinked for nanoparticle formation

- a) Because SB75 (low molecular weight gelatin, confirmed from SEC analysis, Table 3) and VB275 (high molecular weight gelatin, confirmed from SEC analysis, Table 3) produced the same average  $D_H$  (Table 1), we assumed the number of gelatin chains that can be crosslinked to form a nanoparticle was approximately similar.
- b) The average  $D_H$  for SB75 and VB275 was 71 nm. Using the  $R_{\text{minimum}}$  values calculated from SEC (Table 3), the number of gelatin chains for SB75 and VB275 was calculated as follows:

$$x_{\text{SB75\_gelatin chains}} = 71 \text{ nm} / (2.13 * 2 \text{ nm}) = 16.7 = 17$$

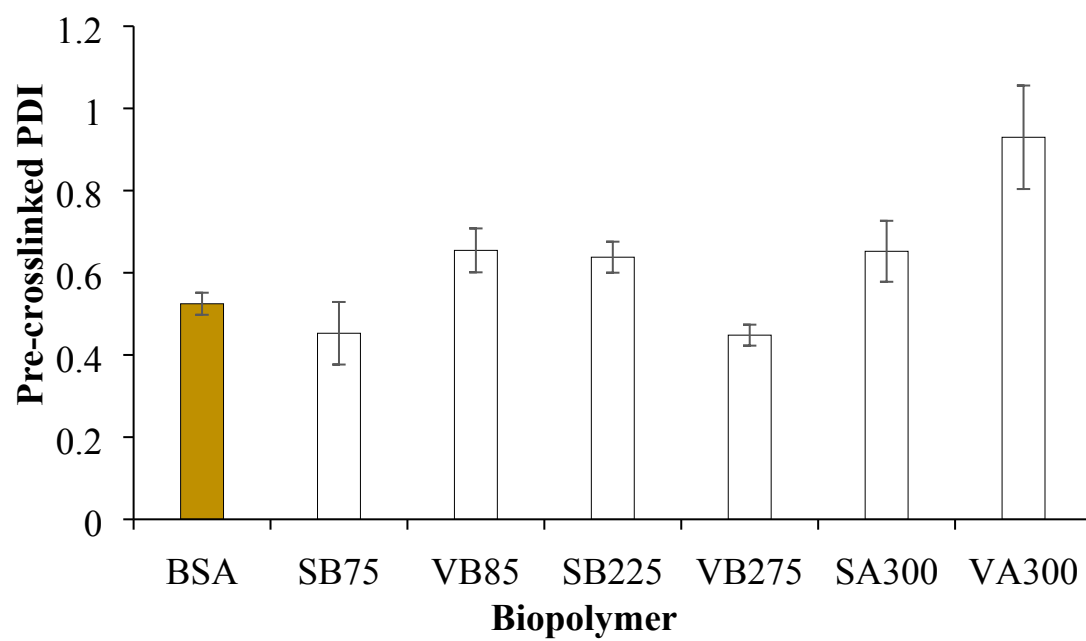
$$x_{\text{VB275\_gelatin chains}} = 71 \text{ nm} / (2.73 * 2 \text{ nm}) = 13.0 = 13$$

- c) Glutaraldehyde can crosslink between 13 and 17 gelatin chain aggregates. Theoretical nanoparticle diameters were calculated (Table 3) for each gelatin using 13 and 17 chains.



**Figure S6.** BSA Macroscale Characterization. (A) Circular dichroism confirms BSA structural integrity revealing alpha helix morphology.





**Figure S7.** Complete biopolymer pre-crosslinked PDI bar graph comparing BSA (shaded) to gelatin (white). Data represent mean  $\pm$  standard deviation.

## Reference

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