Title

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RESEARCH ARTICLE

Formulation and Engineering of Biomaterials

Tangential flow filtration facilitated fractionation of polymerized human serum albumin: Insights into the effects of molecular size on biophysical properties

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Abstract

Human serum albumin (HSA) is currently used as a plasma expander (PE) to increase blood volume during hypovolemic conditions, such as blood loss. However, its effectiveness is suboptimal in septic shock and burn patients due to their enhanced endothelial permeability, resulting in HSA extravasation into the tissue space leading to edema, and deposition of toxic HSA-bound metabolites. Hence, to expand HSA's applicability toward treating patients with compromised endothelial permeability, HSA has been previously polymerized to increase its molecular size thus compartmentalizing the polymerized HSA (PolyHSA) molecules in the vascular space. Previous studies bracketed PolyHSA between 100 kDa and 0.2 μm. In this research, PolyHSA was synthesized at two cross-link densities 43:1 and 60:1 (i.e., molar ratios of glutaraldehyde to HSA) and subsequently fractionated via tangential flow filtration (TFF) into two narrower brackets: bracket A (500 kDa and 0.2 μm) and bracket B (50–500 kDa). PolyHSA within the same size bracket at different cross-link densities exhibited similar solution viscosity, zeta potential, and osmolality but differed in hydrodynamic diameter. At the same cross-link density, the PolyHSA A bracket showed higher viscosity, lowered zeta potential, and a larger hydrodynamic diameter compared with the PolyHSA B bracket while maintaining osmolality. Interestingly, PolyHSA 43:1 B, PolyHSA 60:1 A, and PolyHSA 60:1 B brackets exhibited colloid osmotic pressure similar to HSA, indicating their potential to serve as PEs.

KEYWORDS
colloid osmotic pressure, endothelial dysfunction, human serum albumin, plasma expander, polymerized human serum albumin

1 | INTRODUCTION

Plasma expanders (PEs) are typically administered to patients suffering from hypovolemia in order to replace lost blood volume and increase blood colloid osmotic pressure (COP), which pulls fluid volume from the extravascular space into the vascular space and further expands the blood volume, keeping blood vessels in an open state, which facilitates tissue perfusion.[1] PEs can be subdivided into two classes: crystalloids and colloids.[2] Crystalloids consist of fluids containing electrolytes and other small molecules, and are transient species in the circulation due to capillary leakage.[3] Alternatively, nonprotein, synthetic colloids such as hydroxyethyl starch (HES), which are larger in size compared with the molecules in crystalloid solutions reduce capillary leakage.[4] However, hyperviscous urine was observed upon transfusion of high molecular weight (MW) HES which led to renal failure, while low MW HES can elicit an allergic reaction similar to gelatin.[5] Dextran has similar negative side-effects, and also inhibits blood coagulation.[6]

Since human serum albumin (HSA) accounts for ~80% of the blood COP, it is a naturally occurring PE.[7] HSA also possesses antioxidant properties that reduces inflammation when used as a resuscitation fluid.[8,9] One study demonstrated that intensive care unit administration of either 4% HSA or saline resulted in the same 28 day-mortality rate; however, the type of resuscitation fluid becomes more important when treating hypovolemic patients.[10] Such patients typically suffer from ischemia–reperfusion injury, septic shock, or burns that dysregulate endothelial permeability.[11] HSA administered to patients with compromised endothelial function increased vascular permeability, and promoted HSA extravasation into the tissue space.[12] In this scenario, tissues are directly exposed to toxic HSA-bound metabolites, blood COP decreases, and tissue edema becomes prevalent.[13]

In order to increase the molecular diameter of HSA to prevent tissue extravasation, HSA and hemoglobin were chemically cross-linked to produce core–shell structured protein clusters to serve as a potential artificial oxygen carrier.[14] Another strategy to increase the molecular diameter of HSA relies on chemical cross-linking of HSA. Previously, glutaraldehyde (GA) was used to polymerize HSA into polymerized HSA (PolyHSA), which dually increased the molecular diameter of HSA and increased solution viscosity.[15] The previously synthesized PolyHSA was bracketed between 100 kDa and 0.2 μm and contained some free HSA.[16] Therefore, the goal of this current study was to fine tune the size distribution of PolyHSA molecules via tangential flow filtration (TFF) facilitated fractionation of PolyHSA into two size brackets and to evaluate the biophysical properties of the fractions. The first PolyHSA bracket was fractionated between 500 kDa and 0.2 μm (bracket A), while the second bracket was fractionated between 50 and 500 kDa (bracket B). The hydrodynamic diameter, zeta potential, osmolality, viscosity, and COP of the fractionated PolyHSA products were analyzed to determine the in vitro potential of PolyHSA as a PE. Future work will assess the in vivo response toward transfusion of these PolyHSA brackets in animal models with normal and impaired endothelial function.

2 | MATERIALS AND METHODS

2.1 | Materials

Glutaraldehyde ($C_5H_8O_2$) (70%), sodium chloride (NaCl), potassium chloride (KCl), sodium hydroxide (NaOH), calcium chloride ($CaCl_2{\cdot}2H_2O$), sodium lactate ($NaC_3H_5O_3$), N-acetyl-L-cysteine ($C_5H_9NO_3S$), sodium cyanoborohydride ($NaCNBH_3$), sodium phosphate dibasic (Na_2HPO_4), and sodium phosphate monobasic (NaH_2PO_4) were purchased from Sigma Aldrich (St. Louis, MO). Polyethersulfone (PES) hollow fiber TFF modules with a pore size of 0.2 μm and polysulfone (PS) TFF modules with pore sizes of 500 and 50 kDa were purchased from Repligen (Rancho Dominguez, CA). HSA (AlbuRx® 25, CSL Behring, King of Prussia, PA) was purchased from The Ohio State Outpatient Pharmacy (Columbus, Ohio).

2.2 | PolyHSA synthesis

HSA was diluted to 25 mg/ mL in phosphate buffered saline (PBS) to achieve a final volume of 1.5 L in a 2 L reactor. GA was added to the HSA reaction vessel at a rate of 2 mL/ min to achieve GA:HSA molar ratios of 43:1 and 60:1. The polymerization reaction was allowed to proceed at 37°C for 3 h and was quenched with sodium cyanoborohydride at a 3:1 molar ratio of sodium cyanoborohydride:GA for 30 min. The reaction vessel was then placed in a 4°C refrigerator overnight. Figure 1a shows the reaction setup and procedural timeline. The PolyHSA was initially filtered through a 0.2 μm PES TFF module, and underwent 10 diafiltrations against a 500 kDa PS TFF module with a modified lactated Ringer's solution as described in the literature.[16] The 500 kDa TFF permeate was then subjected to 10 diafiltrations on a 50 kDa PS TFF module with the modified lactated Ringer's solution. The two PolyHSA fractions polymerized at the same cross-link density were concentrated to above 100 mg/mL and stored at −80°C. Figure 1b shows the TFF separation train. Bracket A consisted of PolyHSA molecules between 500 kDa and 0.2 μm, while bracket B consisted of PolyHSA molecules between 50 and 500 kDa (Figure 1). PolyHSA at each GA:HSA molar ratio was produced in triplicate.

2.3 | PolyHSA concentration

The PolyHSA mass concentration from a known volume of solution was quantified via its' dry mass using a Labconco Freeze dryer (Kansas City, MO). Samples were initially frozen to be placed under vacuum. The frozen water from the PolyHSA solution was then removed via sublimation to enable dry mass determination of PolyHSA at the end of the freeze-drying process (48 h).

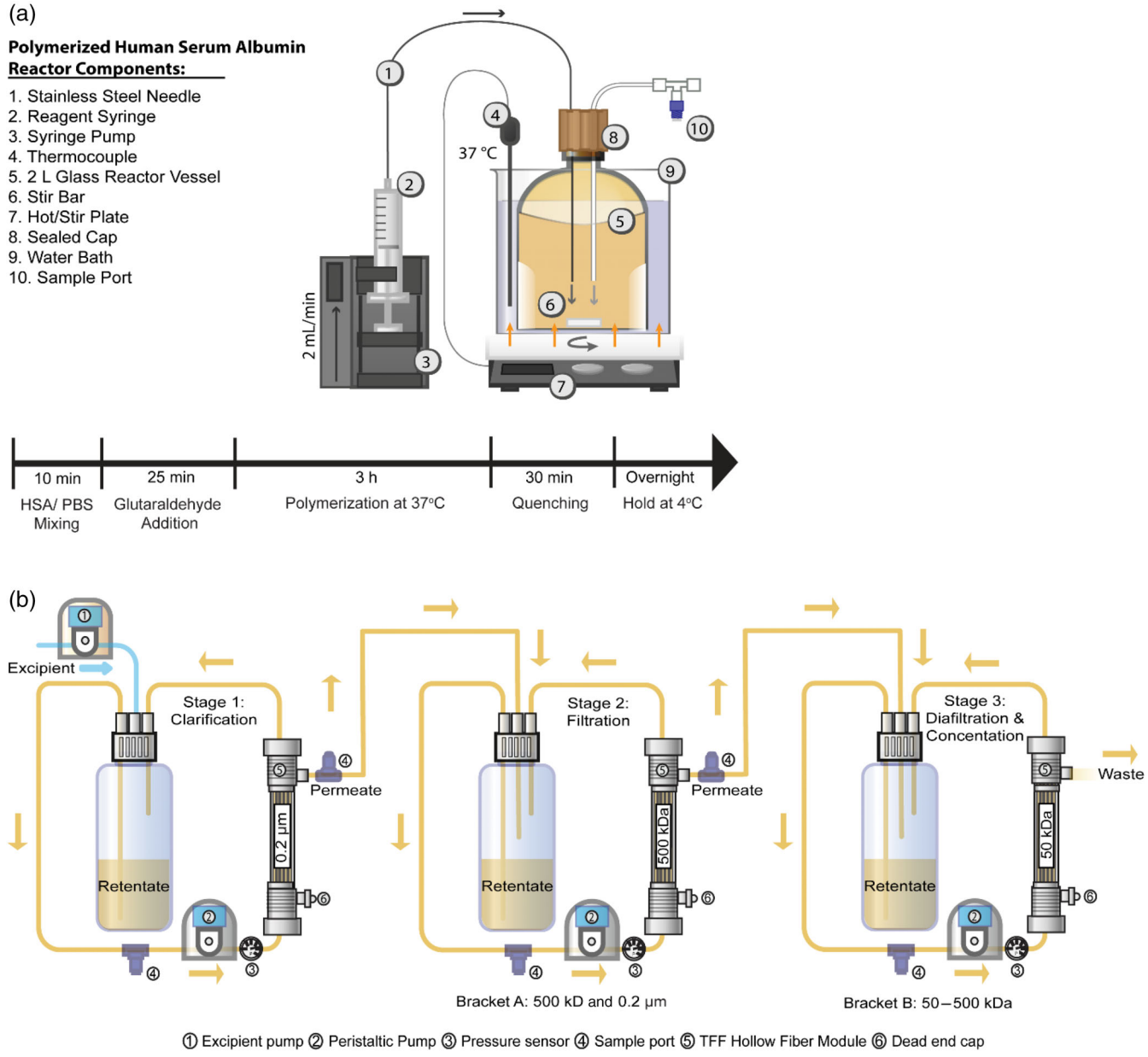

FIGURE 1 (a) Synthesis and (b) fractionation of PolyHSA. HSA, human serum albumin.

2.4 | Size exclusion chromatography elution profile, MW distribution and hydrodynamic diameter

The elution profile of HSA and PolyHSA brackets were measured by size exclusion high-performance liquid chromatography (SEC-HPLC), while the MW distribution of low MW species was assessed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the hydrodynamic diameter was measured via dynamic light scattering (DLS). SEC-HPLC was performed on a SEC-1000 column (ThermoFisher Scientific, Walthan, MA) attached to an Ultimate 3000 HPLC/UHPLC system (ThermoFisher Scientific, Walthan, MA). The Acclaim SEC-1000 column has a 7 µm particle size, 4.6 mm column diameter, and a column length of 300 mm. The absorbance at 280 nm was used to measure total protein. For SDS-PAGE, 2 µg of each sample was mixed in a 1:1 mass ratio with tris-glycine SDS buffer and incubated at 85°C for 20 min. The mixture was then run on a polyacrylamide gel with 12% resolving gel and 4% stacking gel at 225 V (40 min). The gel was stained with Coomassie blue (1 h) and was destained (3×; 1 h) with 70% deionized water, 30% methanol, and 10% acetic acid. The hydrodynamic diameter was measured using a Zetasizer Nano DLS spectrometer (Malvern Instruments, Worcestershire, UK).

2.5 | Zeta potential

The zeta (ξ) potential of HSA and PolyHSA fractions was measured using a zeta potential analyzer (ZetaPALS, Brookhaven, Holtsville, NY).

TABLE 1 Biophysical properties of human serum albumin (HSA) and PolyHSA solutions. Viscosity, colloid osmotic pressure (COP), and osmolality were measured at a total protein concentration of 100 mg/mL. $N = 3$.

Fraction	Hydrodynamic diameter (nm)	Viscosity (cP)	Zeta potential (mV)	Osmolality (osmol/kg)	COP (mmHg)
HSA	5.03 ± 0.15	1.37 ± 0.02	−17.77 ± 0.15	0.293 ± 0.002	41.00 ± 1.00
43:1 A	21.20 ± 0.20	8.95 ± 0.33	−26.08 ± 0.53	0.290 ± 0.005	34.00 ± 1.00
43:1 B	9.87 ± 1.11	5.78 ± 0.17	−21.27 ± 1.62	0.296 ± 0.013	39.67 ± 1.15
60:1 A	29.07 ± 4.21	9.32 ± 0.80	−28.97 ± 2.48	0.280 ± 0.010	38.33 ± 3.05
60:1 B	12.00 ± 2.05	5.66 ± 0.50	−19.68 ± 2.60	0.280 ± 0.004	40.00 ± 1.00

2.6 | Osmolality

The osmolality of HSA and PolyHSA solutions at a total protein concentration of 100 mg/mL was measured using an osmometer based on the freezing point depression method (OSMOMAT 030, Berlin, Germany).

2.7 | Viscosity

The viscosity of HSA and PolyHSA solutions at a total protein concentration of 100 mg/mL was measured using a Brookfield DV3T rheometer with a CP-40 spindle (Brookfield, Middleboro, MA) at a shear rate of 160 s^{-1}. The material was allowed to equilibrate at 37°C for 5 min before the measurement was taken.

2.8 | Colloid osmotic pressure

The COP of HSA and PolyHSA solutions was measured at 100 mg/mL using a Wescor 4420 Colloid Osmometer (Wescor, Logan, UT).

2.9 | Circular dichroism

The secondary structure of HSA and PolyHSA was assessed via circular dichroism (CD) on a JASCO J-1500 CD spectrometer (JASCO, Easton, MD). Samples were prepared at 3 μM, and measured between 200 and 250 nm for changes in secondary structure at 25°C. Protein secondary structure, that is α-helical content, was assessed at the 222 nm minima prior to and after protein polymerization.

2.10 | Data analysis

In this study, all statistical analysis was performed using the Tukey HSD test. Statistical significance was established if $p < 0.05$. Additionally, all results were presented as the mean ± SD, and all statistical analyses were performed using JMP Pro v 16 (Cary, NC). Comparisons were made between (1) unmodified HSA and all PolyHSA brackets; (2) the same PolyHSA bracket at the two different cross-link densities; and (3) the two PolyHSA brackets synthesized at the same cross-link density.

3 | RESULTS

The biophysical properties of HSA and PolyHSA solutions is summarized in Table 1.

3.1 | Size exclusion high-performance liquid chromatography

The elution profile of HSA and PolyHSA brackets was analyzed using SEC-HPLC and the results are shown in Figure 2. HSA was used as a control, and eluted at 9.48 min. PolyHSA 43:1 A had an elution time of 8.17 min, while PolyHSA 60:1 A eluted at 7.92 min. Fraction B at both GA:HSA molar ratios displayed two elution peaks. The two observed elution peaks for PolyHSA 43:1 B occurred at 9.05 and 8.76 min, while PolyHSA 60:1 B eluted at 8.98 and 8.64 min. The A and B fractions of PolyHSA 43:1 and PolyHSA 60:1 contain a minor peak eluting at 13.33 min, which is attributed to the stabilizing amino acid, tryptophan, that was formulated into the manufactured HSA. A vial of HSA containing tryptophan was buffer exchanged with PBS using a 50 kDa TFF module for 10 diacycles to remove tryptophan from the HSA solution. The original HSA solution containing tryptophan and the washed HSA were both analyzed via SEC-HPLC as shown in Figure 2c.

3.2 | SDS-PAGE

HSA and PolyHSA were analyzed via SDS-PAGE as shown in Figure 3. The polymerization of HSA at both cross-linking densities and fractionation into the two sized brackets is demonstrated by the smearing of the PolyHSA lanes in the gel. Additionally, the HSA control has a strong band above the 55 kDa MW standard, which is close to its actual MW (~67 kDa). A similar band is shown in the PolyHSA B fractions. Furthermore, PolyHSA molecules in fraction A are significantly larger than PolyHSA molecules in fraction B, when synthesized at the same molar ratio of GA:HSA.

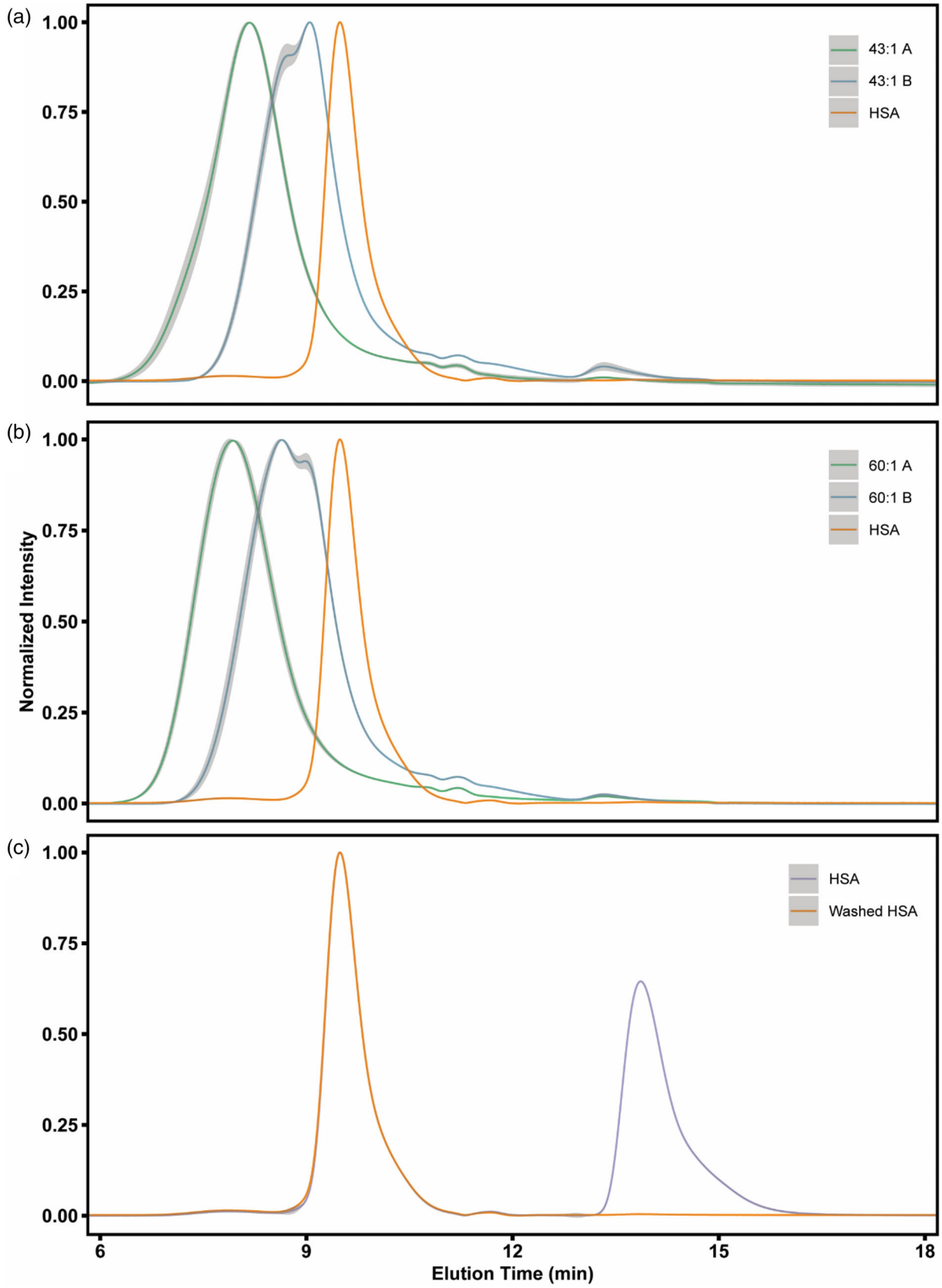

FIGURE 2 Size exclusion high-performance liquid chromatography analysis of human serum albumin (HSA) and PolyHSA. (a) PolyHSA 43:1, (b) PolyHSA 60:1, and (c) HSA with and without tryptophan. The gray shaded areas represent the standard deviation from triplicate HSA and PolyHSA preparations. $N = 3$.

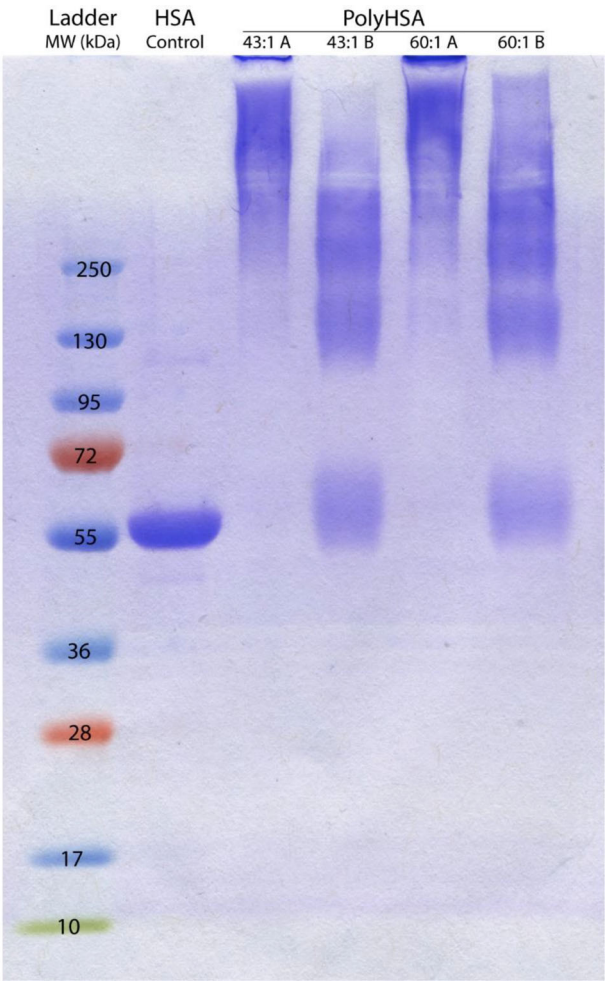

FIGURE 3 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of representative human serum albumin (HSA) and PolyHSA fractions.

3.3 | Dynamic light scattering

DLS measurements indicate a statistically significant increase in the HSA hydrodynamic diameter from 5.03 ± 0.15 to 29.07 ± 4.21 nm ($p < 0.05$) and 12.00 ± 2.05 nm ($p < 0.05$) for PolyHSA 60:1 A and PolyHSA 60:1 B, respectively (Figure 4). Similarly, PolyHSA 43:1 A displayed a statistically significant increased hydrodynamic diameter of 21.20 ± 0.20 nm compared with HSA ($p < 0.05$). However, no significance was observed between PolyHSA 43:1 B and HSA. PolyHSA 43:1 A and PolyHSA 60:1 A are statistically significant in their diameter ($p < 0.05$). At both fixed GA:HSA molar ratios, the A fraction was statistically, significantly larger than the B fraction ($p < 0.05$). Additionally, the polydispersity index of all PolyHSA fractions was less than 0.2.

3.4 | Zeta potential

Insights into the potential colloidal stability of PolyHSA were obtained by measuring the zeta potential and are shown in Figure 5. HSA has a zeta potential of −17.77 ± 0.15 mV, and polymerization statistically decreased the zeta potential to −28.97 ± 2.48 mV ($p < 0.05$) and −26.08 ± 0.53 mV ($p < 0.05$) for PolyHSA 60:1 A and PolyHSA 43:1 A, respectively. The PolyHSA B fraction exists between the HSA control and the higher MW PolyHSA A fraction: −19.68 ± 2.60 mV and −21.27 ± 1.62 mV for PolyHSA 60:1 B and PolyHSA 43:1 B, respectively. However, there was no statistical difference in the zeta potential between either PolyHSA B fractions or the HSA control ($p < 0.05$). On the contrary, the A and B fractions at the same GA:HSA molar ratio are significantly different from one another ($p < 0.05$).

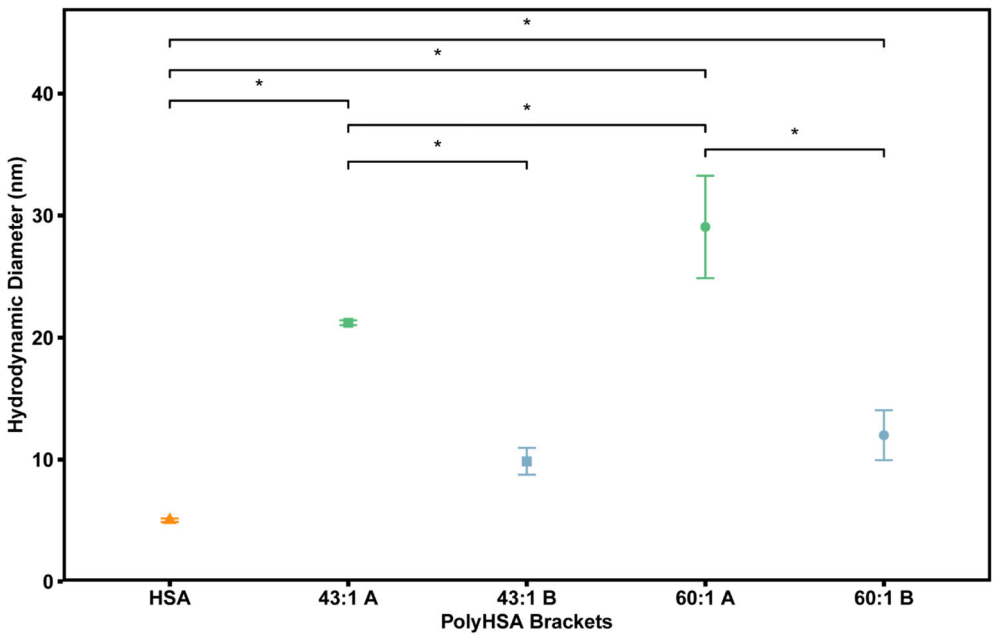

FIGURE 4 Hydrodynamic diameter measured via dynamic light scattering (DLS) for human serum albumin (HSA) and PolyHSA fractions.
$N = 3$, $^*p < 0.05$.

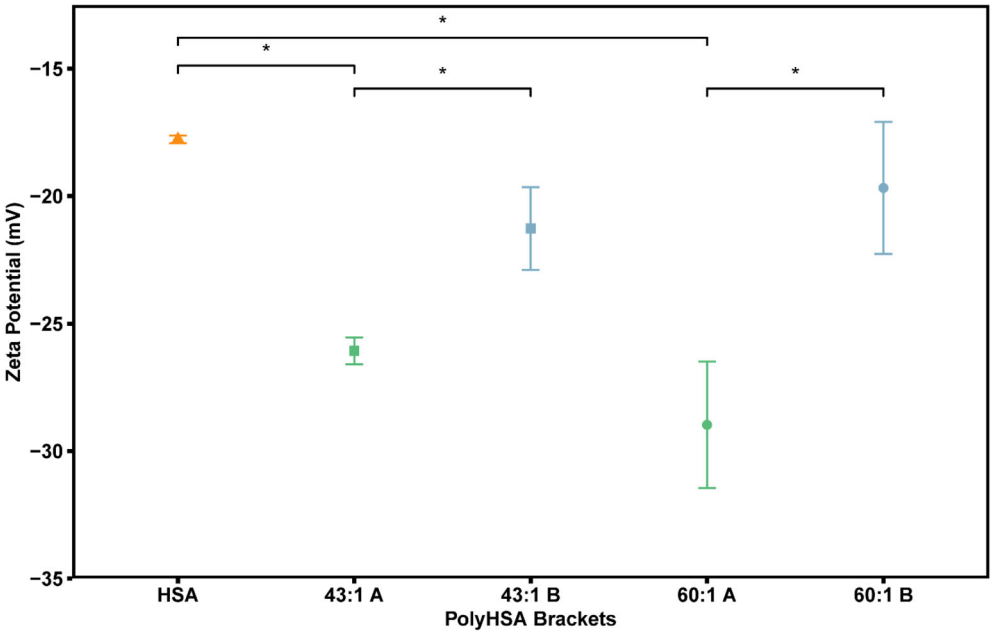

FIGURE 5 Zeta potential of human serum albumin (HSA) and PolyHSA fractions. $N = 3$, $^*p < 0.05$.

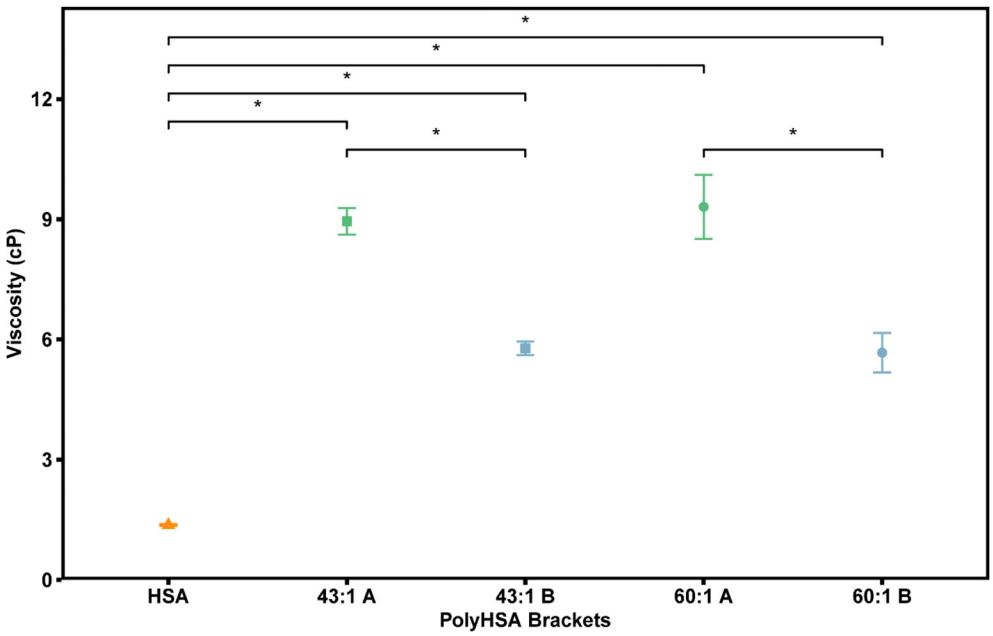

FIGURE 6 Viscosity of human serum albumin (HSA) and PolyHSA brackets. The viscosity was measured at a total protein concentration of 100 mg/mL. $N = 3$, $^*p < 0.05$.

3.5 | Viscosity

As a result of polymerizing HSA, the PolyHSA solution viscosity significantly increased compared to HSA at a fixed total protein concentration of 100 mg/mL for all PolyHSA brackets ($p < 0.05$) as shown in Figure 6. At the same GA:HSA molar ratio, the viscosity of PolyHSA fraction A was significantly higher than PolyHSA fraction B ($p < 0.05$). There was no significant difference in viscosity between the same PolyHSA sized brackets synthesized at different GA:HSA molar ratios. The viscosity of HSA was measured to be 1.37 ± 0.02 cP. The viscosity of the PolyHSA fraction A at both GA:HSA molar ratios ranged between 8 and 10 cP, and the range for PolyHSA fraction B at both GA:HSA molar ratios was between 5 and 6 cP.

3.6 | Osmolality

The osmolality of the PolyHSA product was measured, and the results are summarized in Figure 7. Upon polymerization, the osmolality of all PolyHSA fractions remained unchanged compared with that of HSA ($p > 0.05$).

3.7 | Colloid osmotic pressure

The COP was measured for all PolyHSA brackets as well as HSA, and the results are shown in Figure 8. The COP of HSA, 41.00 ± 1.00 mmHg, is statistically higher than the PolyHSA 43:1 A bracket,

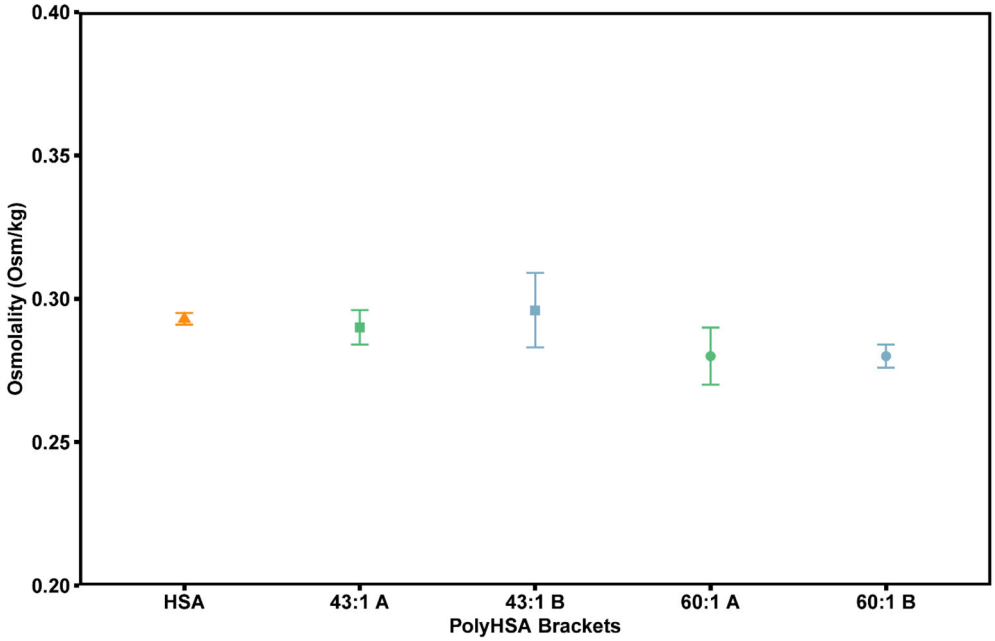

FIGURE 7 Osmolality of human serum albumin (HSA) and PolyHSA brackets. The osmolality was measured at a total protein concentration of 100 mg/mL. $N = 3$.

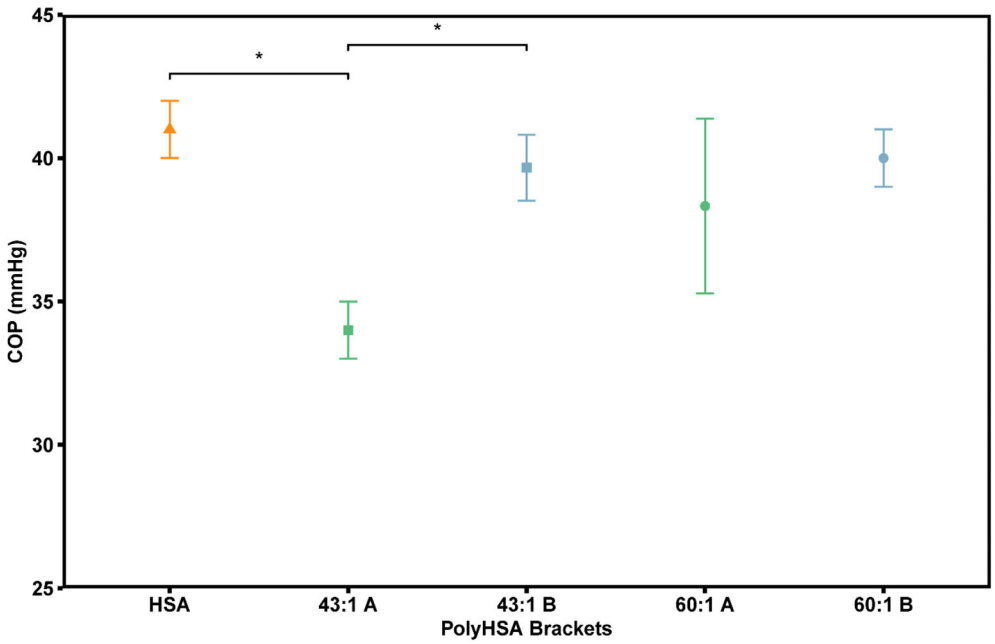

FIGURE 8 Colloid osmotic pressure (COP) for human serum albumin (HSA) and PolyHSA brackets. The COP was measured at a total protein concentration of 100 mg/mL. $N = 3$, $^*p < 0.05$.

34.00 ± 1.00 mmHg, ($p < 0.05$), and that same bracket is significantly lower compared with the PolyHSA 43:1 B bracket, 39.67 ± 1.15, ($p < 0.05$). The COP of PolyHSA 60:1 A and B brackets ranged from an average of 38–40 mmHg.

3.8 | Circular dichroism

Changes in PolyHSA secondary structure (i.e., α-helical content) was assessed via CD spectroscopy. The characteristic signature of α helices consist of two minima at 208 and 222 nm.[17] Figure 9 shows the CD spectra of the PolyHSA brackets and HSA for comparison. Upon polymerization, the minima in the ellipticity decreased for all PolyHSA brackets.

4 | DISCUSSION

A stabilizing amino acid, tryptophan, is formulated into the manufactured HSA solution which is utilized for polymerization.[18] Tryptophan absorbs at the same wavelength as HSA (280 nm), which consequently limited quantification of HSA via UV–visible spectrometry. Therefore, the dry mass approach described in the methods section was used to calculate the final PolyHSA concentration in each

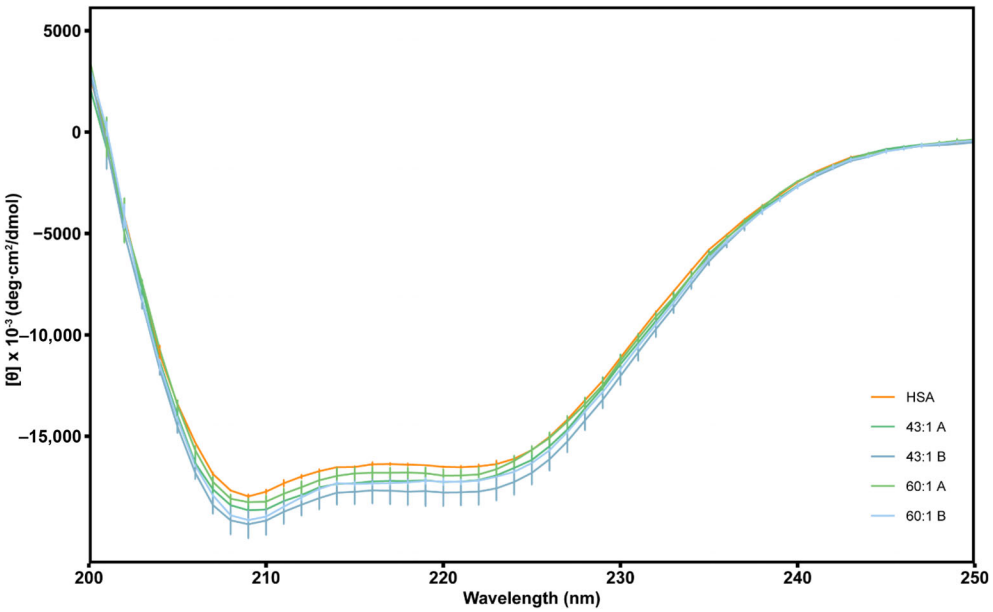

FIGURE 9 Circular dichroism spectroscopy of human serum albumin (HSA) and PolyHSA brackets measured at 3 μM. $N = 3$.

bracket. Figure 2c showcases the prominent SEC-HPLC peak of tryptophan at 13.33 min for the manufactured HSA. The amino acid peak corresponding to tryptophan dramatically decreased in magnitude and was indicative of successful amino acid removal after TFF buffer exchange with PBS as shown not only in Figure 2c, but also in the PolyHSA fractions in Figure 2a,b. It is notable that the HSA precursor for PolyHSA synthesis was the manufacturer's stock solution rather than TFF buffer exchanged HSA. Therefore, tryptophan is not being removed prior to polymerization, but is removed after HSA polymerization via the buffer exchange procedure, which consists of 10 diacycles.

The increase in HSA MW after polymerization was verified by (1) PolyHSA fractions eluting at earlier times compared with unmodified HSA as assessed via SEC-HPLC, (2) retention and smearing of PolyHSA fractions in the SDS-PAGE lanes compared with HSA, and (3) increase in PolyHSA hydrodynamic diameter compared with HSA. The B brackets of PolyHSA at both cross-linking densities were fractionated with a lower MW cutoff (MWCO) TFF module of 50 kDa, which allows HSA molecules (~67 kDa) to be retained in the B fraction. The presence of unmodified HSA is observed in the PolyHSA B fraction via SDS-PAGE analysis in Figure 3. This is further corroborated with the wide, polydisperse SEC-HPLC peaks for the PolyHSA B fractions at 43:1 and 60:1 cross-linking densities in Figure 2a,b, respectively. Additionally, PolyHSA 43:1 B has a higher amount of free HSA compared with PolyHSA 60:1 B, which was verified due to the lack of significance between the hydrodynamic diameter of unmodified HSA and PolyHSA 43:1 B, where significance was present between unmodified HSA and PolyHSA 60:1 B.

As the cross-linking density increases from 43:1 to 60:1, a higher MW HSA polymer is produced. This trend is observed as a decrease in the SEC elution time of both the A and B brackets with increasing GA:HSA molar ratio. An increase in the PolyHSA MW distribution is further reinforced with an increase in the hydrodynamic diameter of PolyHSA with increasing GA:HSA molar ratio. Therefore, SEC-HPLC, SDS-PAGE, and DLS analysis consistently show an increase in the molecular size of HSA upon polymerization. CD analysis shows that the PolyHSA resulted in minor changes in the secondary structure compared with HSA as shown in Figure 9, where it is important to observe the directionality of change. PolyHSA at both cross-linking densities and sized brackets showed a decrease in the α-helical minima in comparison to unmodified HSA, which is indicative of a more stable secondary structure for PolyHSA compared with HSA.[19]

PolyHSA viscosity significantly increases compared with HSA at the same protein concentration due to the increase in PolyHSA molecular size with increasing GA:HSA molar ratio and is shown in Figure 6. This result is expected and can be explained by the increase in molecular interactions and lower dynamics on the molecular scale that results in a more viscous material. This trend aligns with previous viscosity measurements on PolyHSA solutions.[16]

While we have verified the increase in PolyHSA molecular size compared with HSA with increasing GA:HSA molar ratio, it is important to note that certain biophysical properties are independent of size changes such as the zeta potential which is dependent on the protein surface charge distribution. Since HSA has a hydrophilic exterior and a hydrophobic interior, we can infer that polymerizing HSA results in a more polar surface, and thus results in a more negative zeta potential due to the altered topology and increased surface charge. Furthermore, highly charged polymerized proteins prevent aggregation due to electric repulsion and thus make a colloidally stable product for potential biomedical applications.

Lastly, the bracketing of our two cross-linking densities allows the PolyHSA fractions to exhibit COP similar to that of HSA. The lack of statistical significance in COP between HSA and PolyHSA 43:1 B, PolyHSA 60:1 A and PolyHSA 60:1 B brackets is indicative of the ability of those PolyHSA brackets to serve as PEs. Therefore, if these potential PEs with their unaltered COP compared with HSA are

transfused into the circulation, fluid volume will be pulled from the extravascular space into the intravascular space, which will keep the blood vessels open and promote tissue perfusion. However, diluting blood viscosity is not a limiting factor, since these PolyHSA fractions also exhibit significantly higher viscosities compared with HSA. Generally, a viscous PE will increase the wall shear stress on the inner lining of blood vessels, which will promote the endothelium to produce nitric oxide (NO), a vasodilator.[20] In turn, the dilated blood vessels reduce the heart workload, and homeostasis is hypothesized to arise when a PE of normal COP and high viscosity is administered to a sepsis or burn patient.

5 | CONCLUSION

HSA was polymerized at two different GA:HSA molar ratios, and the PolyHSA product was separated into two different size fractions for subsequent characterization of their biophysical properties. For the PolyHSA 43:1 brackets, there was an increase in MW, hydrodynamic diameter, viscosity, along with a more negative zeta potential compared with HSA. The PolyHSA 43:1 A bracket exhibited a statistically significant lower COP than HSA and the PolyHSA 43:1 B bracket. Increasing the GA:HSA molar ratio from 43:1 to 60:1 yielded similar trends. Additionally, PolyHSA 60:1 A and PolyHSA 60:1 B brackets exhibited similar COP compared to HSA. At a fixed GA:HSA molar ratio there was a significant difference in MW, size, viscosity, and zeta potential between the PolyHSA A and B brackets. Therefore, in summary, PolyHSA 43:1 B, PolyHSA 60:1 A and PolyHSA 60:1 B brackets can potentially serve as PEs, since they exhibited similar COPs compared to HSA. In future studies, the ability of the PolyHSA fractions to resuscitate animals during hypovolemic conditions will be evaluated.

AUTHOR CONTRIBUTIONS

Amna Abdalbaqi: Writing – original draft; writing – review and editing; conceptualization; formal analysis; investigation; methodology; visualization. **Ahmad Yahya:** Writing – original draft; conceptualization; investigation; methodology. **Krianthan Govender:** Data curation. **Carlos Muñoz:** Data curation. **Gala Sanchez Van Moer:** Data curation. **Daniela Lucas:** Data curation. **Pedro Cabrales:** Writing – review and editing. **Andre F. Palmer:** Writing – review and editing; conceptualization; resources; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

A provisional patent application was filed based on the materials described in this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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