Albumin Hydrogel Formulation and Potential Biomedical Applications

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Purpose

Albumin is the most abundant plasma protein in humans. It has gained traction as a pharmaceutical excipient due to its biological origin and sustainable availability. It is biodegradable, biocompatible, and a natural xenobiotic carrier in the human body. As a biomaterial, it helps reduces the cytotoxicity of many drugs as a drug delivery vehicle. Our lab is working on the development of personalized formulations using albumin as a biomaterial. Previously, we have shown albumin gel formulation in the presence of salts and thermal shock. It was observed that the gels were produced at high temperatures for a relatively extended period. In this work, we aim to produce albumin hydrogel for drug delivery applications with a reduced temperature or time of exposure. This study shows a systematic characterization to understand the albumin gelling mechanism in the presence of salts as ionic crosslinkers.

Methods

1. Synthesis of Albumin Hydrogels: We have previously established synthesis of albumin gel formation using NaCl salt solution at 85°C for 20 min exposure. In this study, 6%w/v albumin solutions were prepared under variable concentrations of KCl and CaCl2, where gels prepared NaCl solution were used as a control. In this study, we are investigating the effects of batch-to-batch variation of albumin biomaterial, valency (mono- or divalent ions) pH, and salt concentration with the goal of reducing heat exposure (reducing time or temperature).
2. Fluorescence Spectroscopy: 200µL samples were taken in 96 well plates for the measurement of fluorescence quenching. Fluorescence data was collected using the Molecular Devices Spectra Max M3 (s/n: MT 05470).
3. Scanning Electron Microscopy (SEM): Gel synthesized were lyophilized and coated with Au-Pd. Images were captured using the Tescan Mira3 electron microscope. Samples were also used to collect SEM-EDS data.
4. Viscosity Measurement: Viscosity data was collected using a DV2T Brookfield© Viscometer (DV2TLVCJ0), CPA-52Z spindle, and Rheocalc T 1.2.19 software. 0.5 mL of albumin gels were loaded on the stage of the DVT2 Brookfield viscometer. The stage temperature was 25°C. The RPM of the spindle was adjusted to get % torque in 10 to 100% for the final measurements. The graph of relative viscosity was plotted to study the relative sol-gel transition at various time points at 85°C.

Results

1. Synthesis of albumin gels: 6% w/v BSA in 25mM NaCl gelled at 85°C for 20 minutes with varying pH conditions impacting gelling. pH-dependent variability in the gelling of albumin from the same grade but a different batch was observed. The current observation suggests that pH 7.4 is most appropriate for creating injectable albumin gel after heating at 85°C for 20 min. Additional data is needed for a detailed understanding of the pH range required for developing an injectable formulation. The impact of mono and divalent ions on the synthesis of albumin gels was compared. We detected divalent cation gelling of albumin in a few seconds at 85°C, in contrast monovalent ions required 20 min exposure for gelling process.
2. Characterization of Albumin Gels: As divalent cations produce gels faster; this was confirmed by fluorescence spectroscopy where the rate of quenching was taken as a marker for gelling behavior. The gelling rate was also confirmed by using viscosity measurement over time (of heat exposure 85°C) for albumin

solution made using mono and divalent cations. It was observed that dynamic light scattering data shows thermal aggregation mediated ionically crosslinked gels. Hence, scanning electron microscopic images were taken for the formation of albumin gels made using mono and divalent cations. These observations show variations in the structural morphology of the gels produced indicative of variations in the ionic crosslinking behavior.

Conclusion

There is a significant difference in gelling behavior between different batches of bovine serum albumin. We used heat-shock-stabilized BSA for the synthesis of the gel. Our previous observation showed that the use of pH 7.8 produced a transparent injectable gel. A different batch of the same heat-shock-stabilized BSA produces transparent gel at pH 7.4. The present study confirms previous work that changing salt concentration affects gelling behavior. As concentration increases, gel hardness increases. Also, it confirms that changing the salt used from a monovalent salt to a divalent also changes gelling behavior. Divalent ions form a harder, opaque gel in comparison to monovalent ions. However, decreasing the concentration of salt and decreasing the time of exposure to 85°C form a gel similar in nature to monovalent gels. SEM images show that $CaCl_2$ gels are relatively porous. SEM-EDS data confirm the presence of salts in gels that serve as ionic crosslinkers during gel formation.

Keywords: albumin hydrogel, crosslinking, thermal denaturation, drug delivery