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APPLICATION NOTE

AN2005: Characterizing size distributions of broadly heterogeneous proteins with FFF-MALS

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Introduction

Although gelatin provides important advantages for colloidal drug carriers, like its proteinaceous structure and its biodegradability, there is one major drawback of gelatin that disturbs the manufacturing process. As a consequence of the extraction of gelatin from collagen originating from different animal sources, its molecular weight distribution is usually very heterogeneous. Generating gelatin nanoparticles via desolvation is not possible when the material is broadly heterogeneous.

To overcome this problem a two-step desolvation technique was proposed and successfully realized. Part of this improved gelatin manufacturing process is the separation of the gelatin base material into high-molecular weight (HMW) and a low-molecular-weight (LMW) fractions, whereby the HMW fraction is further applied for the production of the gelatin nanoparticles. Determination of molecular weight by field-flow fractionation coupled to multi-angle light scattering (FFF-MALS) was critical to optimizing the final nanoparticles.

Materials and Methods

To determine the molecular weight and molecular weight distribution of the HMW fraction and to understand what had been empirically found for the two-step desolvation process, we used an Eclipse™ FFF system with a DAWN® MALS instrument. The Eclipse—controlled by VISION™ software—serves to separate the gelatin molecules by size; MALS data from the DAWN, combined with a UV absorbance data, provides the molecular weight and size of each eluting fraction. ASTRA® light scattering software is called by VISION to control the DAWN and record data, then calculates the results and converts the fractionation data to molecular weight moments and distributions.

We investigated the gelatin base material we usually purchased, the HMW fraction obtained during the two-step desolvation, and some modified gelatin batches from a new supplier. The concentration of all gelatin batches was 2.5 mg/mL, and 100 µL of solution were injected for each fractionation run.

Results and Discussion

During our experiments we were able to show the differences between the gelatin base material and the HMW fraction, influencing the nanoparticle's formation process (Figure 1). Furthermore, while describing the new modified batches, we were able to define a specific molecular weight and molecular weight distribution response that is necessary to simplify the nanoparticle formation to a one-step desolvation process (Figure 2).

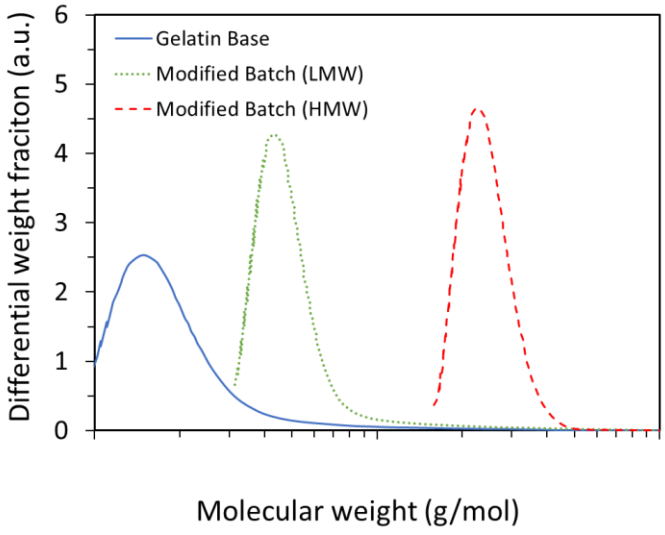

Figure 1. The differences in the molecular weight distribution between the gelatin base and the new HMW fraction are shown. Even the slightly shifted balance point of the new material enables us to produce gelatin nanoparticles by means of a one-step desolvation procedure.

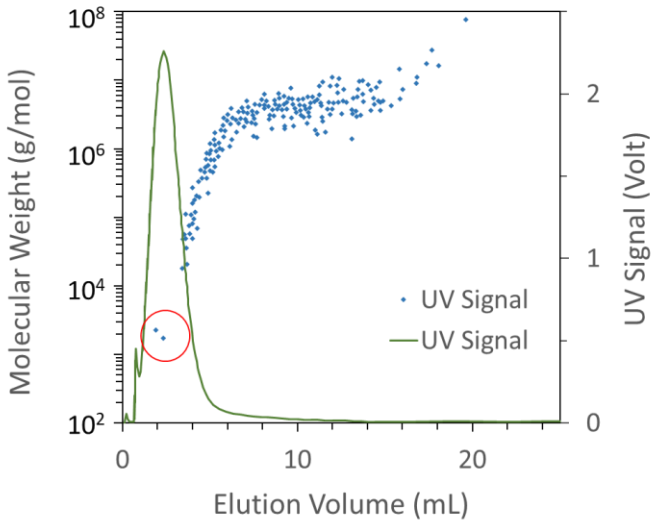

Figure 2. Molecular weight of one of the new experimentally modified gelatin batches (blue squares), overlaid on the UV fractogram (source data for Figure 1, green line). The graph shows the reduced amount of the LMW fraction of this experimentally modified batch (red circle)

Conclusion

Effective separation of HMW protein species, well beyond the capabilities of size-exclusion chromatography, is afforded by an Eclipse-based FFF system. Coupling MALS and UV detectors to FFF enables molecular weight measurements of such species across many orders of magnitude through tens of millions of g/mol. These capabilities are essential for developing protein-based drug delivery nanoparticles.

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